

KINETICS OF ANAEROBIC SULPHATE REDUCTION IN IMMOBILISED CELL BIOREACTORS

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Canada

By
Vikrama Krishnan Baskaran

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ABSTRACT

Many industrial activities discharge sulphate- and metal-containing wastewaters, including the manufacture of pulp and paper, mining and mineral processing, and petrochemical industries. Acid mine drainage (AMD) is an example of such sulphate- and metal-containing waste streams. Formation of AMD is generally the result of uncontrolled oxidation of the sulphide minerals present in the terrain in which the drainage flows with concomitant leaching of the metals. Acid mine drainage (AMD) and other sulphate- and metal-containing waste streams are amenable to active biological treatment. Anaerobic reduction of sulphate, reaction of produced sulphide with metal ions present in the waste stream, and biooxidation of excess sulphide are three main sub-processes involved in the active biotreatment of AMD. Anaerobic reduction of sulphate can be achieved in continuous stirred tank bioreactors with freely suspended cells or in immobilized cell bioreactors. The application of freely suspended cells in a continuous system dictates a high residence time to prevent cell wash-out, unless a biomass recycle stream is used. In an immobilized cell system biomass residence time becomes uncoupled from the hydraulic residence time, thus operation of bioreactor at shorter residence times becomes possible. In the present work, kinetics of anaerobic sulphate reduction was studied in continuous immobilized cell packed-bed bioreactors. Effects of carrier matrix, concentration of sulphate in the feed and sulphate volumetric loading rate on the performance of the bioreactor were investigated. The bioreactor performance, in terms of sulphate reduction rate, was dependent on the nature of the carrier matrix, specifically the total surface area which was provided by the matrix for the establishment of biofilm. Among the three tested carrier matrices, sand displayed the

superior performance and the maximum volumetric reduction rate of 1.7 g/L-h was achieved at the shortest residence time of 0.5 h. This volumetric reduction rate was 40 and 8 folds faster than the volumetric reduction rates obtained with glass beads (0.04 g/L-h; residence time: 28.6 h) and foam BSP (0.2 g/L-h; residence time: 5.3 h), respectively. Further kinetic studies with sand as a carrier matrix indicated that the extent of volumetric reduction rate was dependent on the feed sulphate concentration and volumetric loading rate. At a constant feed sulphate concentration, increases in volumetric loading rate caused the volumetric reduction rate to pass through a maximum, while increases in feed sulphate concentrations from 1.0 g/L to 5.0 g/L led to lower volumetric reduction rates. The maximum volumetric reduction rates achieved in the bioreactors fed with initial sulphate concentration of 1.0, 2.5 and 5.0 g/L were 1.71, 0.82 and 0.68 g/L-h, respectively. The coupling of lactate utilization to sulphate reduction was observed in all experimental runs and the rates calculated based on the experimental data were in close agreement with calculated theoretical rates, using the stoichiometry of the reactions involved. The maximum volumetric reduction rates achieved in the immobilized cell bioreactors were significantly faster than those reported for freely suspended cells employed in the stirred tank bioreactors.

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DEDICATION

This work is dedicated to my parents and sister

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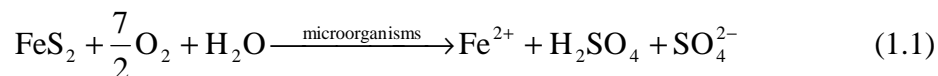
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1. INTRODUCTION

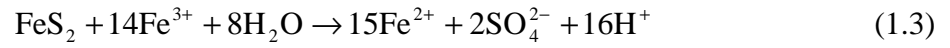
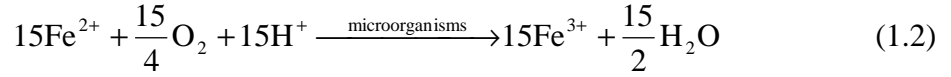
High sulphate containing waste waters are generated from various industrial activities. These include pulp and paper industries, mining and mineral processing, production of explosives, scrubbing of flue gases, food processing and petrochemical industries (Lens et al., 1998).

Canada is one of the largest exporters of mineral ores and as such the environmental problems associated with these mining activities are significant and need attention. For instance, in Canada significant amount of waste rock and tailings are produced in active mining sites. Waste rocks and tailings generated from mining operations usually contain sulphide minerals which due to exposure to air and water are oxidised and release metallic ions, sulphate and acidity. Some of the present microbial species such as *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Thiobacillus caldus* will enhance these oxidation reactions (Garcia et al., 1996). The mechanisms for the production of acid mine drainage by the oxidation of sulphide minerals were proposed by Gray (1997). As a typical example, the oxidation of pyrite ore is discussed here, as shown in reaction 1.1.



The Fe^{2+} ions formed by oxidation of pyrite ore are further oxidised by bacterial species such as *Acidithiobacillus ferrooxidans* to Fe^{3+} ions, and these ferric ions may

also further react with pyrite to form ferrous ions, sulphate and hydrogen ions as given in the reactions 1.2 and 1.3.



Waste rocks and mine tailings could contain a variety of sulphide minerals. Table 1.1 summarizes the different types of sulphide minerals which could be present in the tailings. Most of the sulphide minerals shown in the Table 1.1 could undergo oxidation reactions similar to that of pyrite resulting in increased acidity, sulphate and metal concentrations in the final effluent. This effluent, which contains high amount of sulphate, metal and hydrogen ions is called *Acid Mine Drainage* (AMD). The composition of acid mine drainage differs from place to place and depends on the minerals present. Table 1.2 provides typical composition of AMD streams as reported by different workers.

Table 1.1 Sulphide minerals present in mine tailings (Ferguson and Erickson, 1988)

Minerals	Composition
Pyrite	FeS ₂
Marcasite	FeS ₂
Chalcopyrite	CuFeS ₂
Chalcocite	Cu ₂ S
Sphalerite	ZnS
Galena	PbS
Millerite	NiS
Pyrrhotite	Fe _{1-x} S (where 0 < x < 0.2)
Arsenopyrite	FeAsS
Cinnabar	HgS

Table 1.2 Composition of AMD streams as reported by various workers

	Wallenburg, Norway¹	Anaconda, USA²	Avoca, Ireland³	Bullhouse, England⁴	Rio Tinto, Spain⁵	Wheal Jane, England⁴	Parys Mine, Wales⁴
pH	5.5	2.5	2.7	5.9	2.2	3.4	2.5
SO₄²⁻	2940	3510	10579	-	10000	400	1550
Total Fe	139	300	1031	61	2300	290	650
Zn²⁺	34	155	362	-	225	132	60
Cu²⁺	2	29	243	-	109	1.2	40
Al³⁺	1	125	-	1.2	-	27	70
Mn²⁺	350	88	-	15	-	8	10

All units in mg/L except pH. ¹From Christensen et al. (1996), ²From Jenke and Diebold (1983), ³From Gray (1997), ⁴From Johnson and Hallberg (2003), ⁵From López-Archilla et al. (2001).

1.1. Impacts of Acid Mine Drainage

Acid mine drainage is a serious problem in most provinces of Canada. It has been estimated that 135 mine sites are generating acid mine drainage across Canada (Feasby and Jones, 1994). The Mount Washington Mine, the Britannia Mine and the Equity Silver Mine in British Columbia are examples of these contaminated sites. In the United States, places like the Ohio river valley and the Appalacean regions are vastly affected by the AMD problem (DeNicola and Stapleton, 2002).

The sulphate present in the acid mine drainage can affect the concrete structures (Neville, 2004; Santhanam et al., 2001). Moreover the present sulphate can be reduced to hydrogen sulphide resulting in unfavourable ecological effects. Hydrogen sulphide corrodes copper (Tran et al., 2003), iron (Dinh et al., 2004) and carbon steel (Rao et al., 2000). In addition to these corrosion effects, hydrogen sulphide has a unique odour which is very harmful to the environment. Apart from the sulphate and sulphide, the acid and metallic ions present in the AMD makes its impact more severe. The release of AMD into the natural water bodies has been proved to be harmful to aquatic and plant lives. The acid present in the acid mine drainage corrodes bridges and other infrastructures (Santhanam, 2001). The metal ions present in the acid mine drainage, for example Fe^{3+} , when integrated into the rivers or other water bodies forms iron (III) hydroxide which is a yellow precipitate, called yellow boy. The formation of yellow boy (Figure 1.1) can be described by the following reaction.

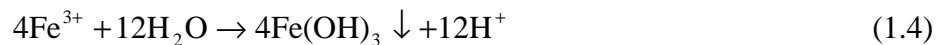




Figure 1.1 Picture of a typical AMD stream

Heavy metals that may be present in the AMD are extremely harmful to human health and can cause nerve disorders, problems associated with lungs and bones, reproductive system, birth defects and some metals are even reported to be carcinogenic (Malik, 2004).

1.2. Overview of Existing Treatment Technologies

Various technologies have been proposed for the treatment of AMD. The treatment processes can be classified into two categories: passive and active processes. Passive treatment processes commonly replace the conventional neutralisation techniques where alkaline reagents such as lime and limestone are used to neutralise the AMD. In some passive treatment technologies the naturally occurring chemical and biological process aid the treatment of acid mine drainage. Passive treatment processes require less energy and chemicals and the maintenance cost is relatively low. Moreover,

operation of these processes does not require extensive manpower. A large number of passive treatment technologies are employed for the treatment of AMD. These include natural wetlands, aerobic and anaerobic wetlands, open limestone channels, diversion wells, successive alkalinity producing systems, anoxic limestone drains and limestone ponds. The passive processes are considered low-cost treatment technologies. However, the efficiency of the process is low and a large land requirement is one of the main drawbacks.

Active treatment processes are highly efficient when compared to the passive treatment processes. Active treatment processes makes use of same chemical and biological reactions as in passive processes, but in a controlled environment. The efficiency of treatment is usually improved through the application of energy, chemical and biological agents. Active treatment processes require higher maintenance cost and manpower when compared to the passive processes. But these costs are offset by the high efficiency of the processes. In the following sections various passive and active treatment processes are described briefly.

1.2.1. Passive Treatment Processes

1.2.1.1. Natural Wetlands

Some species of plants belonging to the genera *Sphagnum*, *Typha*, *Juncus*, *Scirpus* and *Proserpinaca* are reported to be efficient in the treatment of acid mine drainage through the uptake of metals (Woulds and Ngwenya, 2004; Mays and Edwards, 2001). Treatment of AMD in natural wetland is a very slow process and requires large

land area. After a short period of exposure to AMD, the plants began to die, due to the presence of high levels of metals.

1.2.1.2. Aerobic Wetlands

These are basically man made wetlands which are constructed by excavating the land and filling it up with clay and soil (0.3-1.0 m deep) to support the growth of wetland plants (Figure 1.2). The AMD flows horizontally in these wetlands.

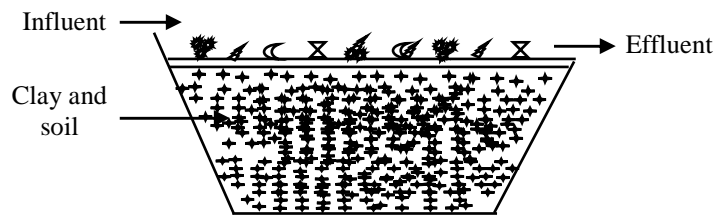
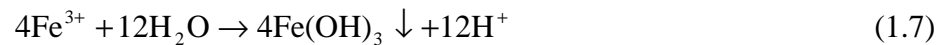
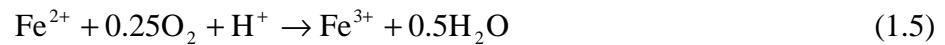


Figure 1.2 Aerobic wetlands (Mitra and Saracoglu, 2004)

The low flow rate of AMD into the wetland provides high residence time and aeration. In this process metals are mainly removed by oxidation and precipitation as metal oxyhydroxides and metal hydroxides. The mechanism is illustrated in reactions (1.5), (1.6) and (1.7) using iron as an example of metallic ion:



The wetland plants also contribute in increasing the organic content by secretion and degradation. Apart from precipitation, removal of metal also takes place in small quantities by biological sulphate reduction and precipitation of the metals as metal

sulphides, as well as uptake by plants in small quantities (Mays and Edwards, 2001). The disadvantages of the aerobic wetland process is that it decreases the pH of the AMD as it releases H^+ ions during precipitation of metals as shown in reactions (1.6) and (1.7). Aerobic wetland is also a very slow process and needs a large land area.

1.2.1.3. Anaerobic Wetlands

Anaerobic wetland shown in Figure (1.3) is a man-made constructed wetland. Here limestone is either mixed with organic matter or laid under the organic matter (Collins et al., 2004). The limestone present imparts alkalinity to the AMD whereas the organic matter encourages the growth of wetland plants and sulphate reducing bacteria. These bacteria generate additional alkalinity by oxidising the organic matter and utilising that energy for sulphate reduction. This mechanism produces bicarbonate ion and sulphide. The reactions for the release of alkalinity by limestone and sulphate reduction, in the presence of acetate as an organic compound, are given by reactions (1.8) and (1.9).

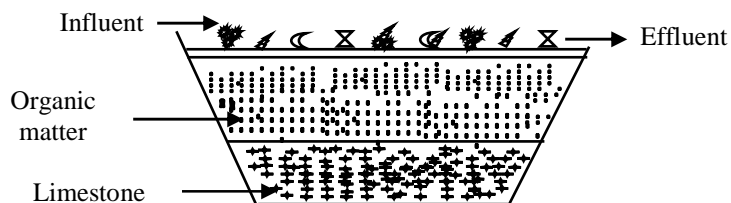
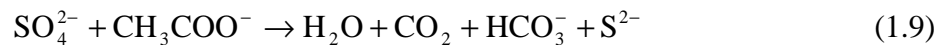


Figure 1.3 Anaerobic wetlands (Mitra and Saracoglu, 2004)

When the AMD passes through the organic matter it becomes anoxic which in turn supports the growth of sulphate reducing bacteria (SRB) and production of sulphide. Due to the prevalence of anaerobic conditions the oxidation of metal ions is quite low and thereby the formation of metal oxyhydroxide is lower when compared to aerobic wetlands. Metals are removed as precipitated metal sulphides, uptake by plants, sorption into organic matter and as metal hydroxides and oxyhydroxides (Woulds and Ngwenya, 2004). The disadvantage of anaerobic wetland is the armouring of limestone with the metal hydroxide and oxyhydroxide precipitates and gypsum. The other disadvantage is the inefficient mixing of the limestone with the AMD present at the surface. Similar to aerobic wetland, a high residence time and large land area is required for this approach.

1.2.1.4. Anoxic Limestone Drains

In anoxic limestone drains, limestone is buried or placed inside pipes or covered by plastic liners and the anoxic AMD is passed through the limestone bed (Figure 1.4). It is important that the waste water under treatment to be maintained anoxic, otherwise the metals present in the AMD will oxidise and form metal hydroxide and oxyhydroxide precipitates. These precipitates will form a coating on the limestone thereby reducing the alkalinity producing potential of the limestone and possibly result in clogging or blockage of the system (Robbins et al., 1999).

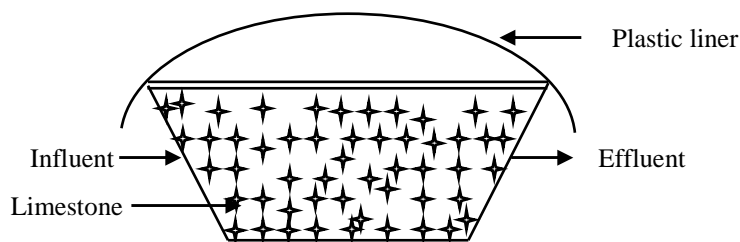


Figure 1.4 Anoxic limestone drains (Mitra and Saracoglu, 2004)

The anoxic limestone drain process was basically designed to introduce the alkalinity to the AMD by the same mechanism as shown in reaction (1.8). When the treated AMD leaves the anoxic limestone drains it is exposed to the aerobic conditions in settling ponds where the metals are oxidised and precipitated as metal hydroxide and oxyhydroxides. Since AMD in general contains high level of dissolved oxygen it is advisable that the AMD is passed through an organic carbon material to remove the dissolved oxygen or the anoxic limestone drains should be built as close as possible to the point of discharge of the AMD.

1.2.1.5. Successive Alkalinity Producing Systems

Successive alkalinity producing systems are a modified form of anoxic limestone drains in which the limestone bed is at the bottom and the organic material is spread over it (Figure 1.5). The high oxygen content of the AMD is reduced or entirely removed by the help of the organic material. This anoxic AMD passes through the limestone which will impart alkalinity to the AMD (Johnson and Hallberg, 2005). Pipes are provided at the bottom of the limestone bed which will transfer the alkaline waste

water to an aerobic system where the metals will be oxidised and precipitated. The AMD is driven through the pipes by means of hydraulic head.

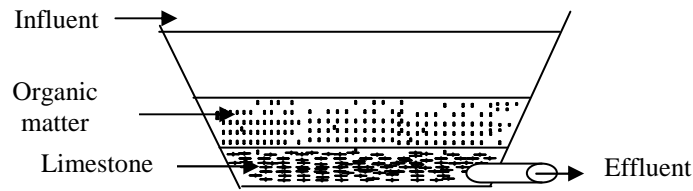


Figure 1.5 Successive alkalinity producing systems (Johnson and Hallberg, 2005)

1.2.1.6. Open Limestone Ponds

In open limestone ponds, limestone is placed in a pond which is fed with AMD with a residence time of 1-2 days. It was reported if open limestone drains were adopted for the treatment of AMD, the overall process would be cheaper when compared with anoxic limestone drains (Cravotta and Trahan, 1999). The advantage of this process is that the limestone is not buried so it is easy to determine if the limestone is coated with a layer of metal hydroxide precipitates or exhausted due to dissolution. In the first case the removal of precipitates is achieved by mechanically disturbing the limestone present in the pond and in the second case fresh limestone is added to the pond.

1.2.1.7. Open Limestone Channels

Figure 1.6 shows a constructed open limestone channel where limestone is placed at the bottom and at the sides of the channels through which the AMD passes (Ziemkiewicz et al., 1997). Constructing the channels with a slope of 20% results in better efficiency as the velocity and turbulence of the AMD will be high which would

wash away the precipitates coated over the limestone and will keep the metal hydroxide precipitate suspended in the AMD.

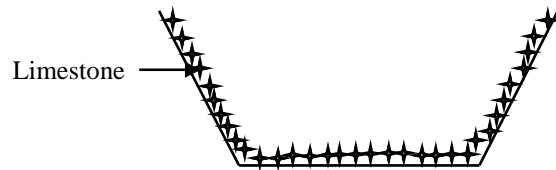


Figure 1.6 Open limestone channel (Mitra and Saracoglu, 2004)

1.2.1.8. Diversion Wells

Diversion well as shown in Figure 1.7 is a metal or concrete tank with diameter of 1.5-1.8 m and depth of 2-2.5 m which is filled with crushed limestone (Ridge and Seif, 2002). It is located near the origin of an AMD stream and in some cases it is buried to provide the necessary head for the flow of AMD. A pipe with a diameter 20-30 cm is passed through the middle of the tank which ends shortly above the bottom of the tank.

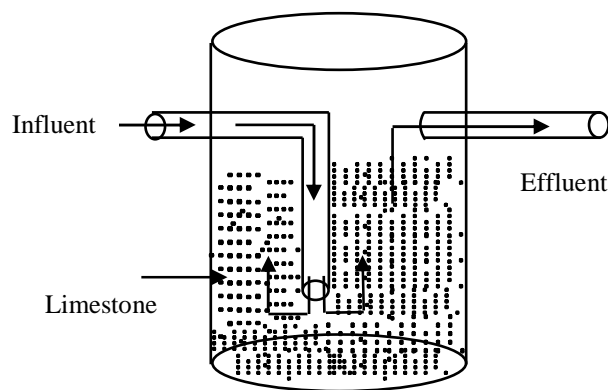


Figure 1.7 Diversion wells (Ridge and Seif, 2002)

The AMD is collected from the upstream through a pipe and is transferred with the help of hydraulic head existing in the stream. The water flows through the pipe and reaches the bottom then moves upward through the limestone bed and leaves the well through the exit provided at the top. The velocity of the AMD should be high such that it fluidises the limestone bed. This helps in better dissolution of limestone and the removal of metal hydroxides and oxyhydroxides. The metal precipitates that are formed are removed from the treated AMD by the use of a settling pond.

Although the passive treatment technology is cheap, the efficiency of the processes is generally very low and due to that reason the land required to treat the acid mine drainage is large. Also the sulphide which is released, due to the reduction of sulphate, will create odour and toxic problems in the environment if not treated. It was reported that about one tonne of sludge is formed per tonne of limestone used. This sludge contains about 95-98% water and harmful elements which makes dewatering and handling extremely expensive and tedious (Bunce et al., 2001).

1.2.2. Active Treatment Processes

Technologies such as reverse osmosis, ion exchange, limestone and chemical neutralisation and active biological treatment fall under the category of active treatment processes. Research studies regarding the treatment of AMD using reverse osmosis and ion exchange are in the seminal stage and no large scale plant, based on these treatments, exists. On the other hand many full scale plants exist for the treatment of acid mine drainage by limestone and chemical neutralisation and a few in which active biological treatment is used.

1.2.2.1. Limestone and Chemical Neutralisation

The process of treating the acid mine drainage with limestone and other chemicals such as hydrated lime, sodium carbonate (soda ash) and sodium hydroxide (caustic soda) is a common technique adopted by the mining industries. For these systems care should be taken for the proper mixing of the chemicals with the acid mine drainage, and the chemicals used should be in the crushed form to increase the surface area and thereby rate of chemicals dissociation. These chemicals are used to increase the alkalinity of the acid mine drainage which will be followed by aeration or peroxide addition to oxidise and precipitate the metals as metal hydroxides and oxyhydroxides. Lime and limestone are cheap while the cost of other neutralising agents such as soda ash, caustic soda and ammonia are high. The major disadvantage of using lime and limestone is that after a certain period of reaction, the surface of particles becomes coated with metal hydroxides and oxyhydroxides and gypsum which will decrease the process efficiency. It has been reported that the use of CO₂ in a pulsed limestone bed reactor improves the efficiency of the reactor by decreasing the armouring of limestone with metal oxyhydroxides when compared to the continuous flow system without CO₂ (Hammarstrom et al., 2003). The other disadvantage of using these chemicals is the production of sludge and associated problems of disposing these secondary wastes. Also the chemicals offer weak bonding forces with the heavy metals and the metal precipitates formed may dissociate into metal ions. It has been reported that 2,6-pyridinediamidoethanethiol (PyDET) is capable of forming an irreversible bond with the metal and the precipitate is stable at a pH range of 0.0 to 14.0 (Matlock et al., 2002). It was also reported that PyDET was able to reduce mercury and lead concentrations from

50ppm to 0.1ppm and 0.05ppm, respectively. The addition of PyDET can be used as a pre-treatment step to remove the metals from the AMD before treating it with chemicals for further neutralisation. However, this has not been implemented in a large scale considering the cost associated with the process.

1.2.2.2. Active Biological Treatment

Biological treatment of waste waters has been widely adopted in the industry as an efficient technology. Acid mine drainage is also amenable for active biological treatment and the treatment process involves three steps as shown in Figure 1.8. Sulphate reducing bacteria (SRB) when provided with a proper carbon and energy source reduce the sulphate content of AMD to sulphide. The produced sulphide then reacts with metal ions, if present, and precipitates as metal sulphides, which can be processed for the recovery of valuable metals. In the absence of sufficient metal ions a further step for the treatment of excess sulphide is required. This is achieved using sulphide-oxidizing bacteria (SOB), which utilize either oxygen or nitrate as an electron acceptor for oxidation of sulphide to elemental sulphur. The focus of the present research was on anaerobic sulphate reduction and thus important information on sulphate reducing bacteria and anaerobic sulphate reduction is reviewed in the following sections.

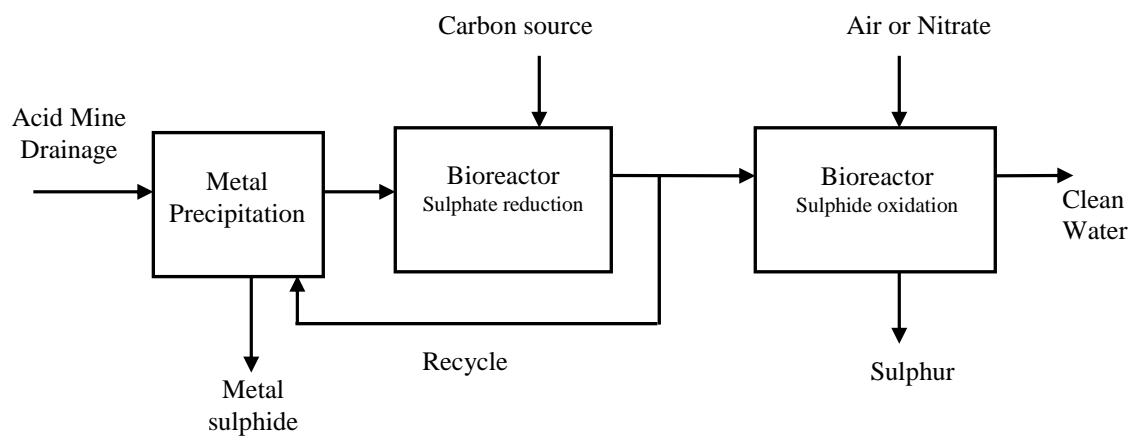


Figure 1.8 Active biological treatment for acid mine drainage and other sulphate and metal containing waste streams

2. LITERATURE REVIEW

In this section of thesis, some important aspects of anaerobic sulphate reduction, including information on various species of sulphate reducing bacteria (SRB), environmental factors affecting the growth and activity of SRB, kinetics of anaerobic sulphate reduction and the various types of reactors employed to carry out this reaction are discussed. It is necessary to have an understanding of dissimilatory sulphate reduction, which is a part of the natural sulphur cycle (Figure 2.1). Green plants and some micro-organisms can consume the sulphur in its oxidised state (sulphate) where the sulphur in the sulphate is utilised for the formation of amino acids, proteins, nucleic acids, and various sulphur-containing coenzymes. These amino acids are stored in the form of microbial protein in the plants (Postgate, 1984). When plants are consumed by animals the sulphur present in the microbial protein is virtually returned to the environment in the reduced form (sulphide) by animal excretion and putrefaction of the dead organisms. This process is called assimilatory sulphate reduction. Of more relevance to this research is a second type of sulphate reduction. This is carried out by a unique type of anaerobic micro-organisms called the sulphate reducing bacteria (SRB). SRB reduce sulphate to sulphide in the presence of a carbon source (acetate, lactate, propionate, etc.) and the sulphide produced can be oxidised to sulphate via

elemental sulphur by sulphide oxidising bacteria (SOB) in the presence of air or nitrate.

This process is called dissimilatory sulphate reduction.

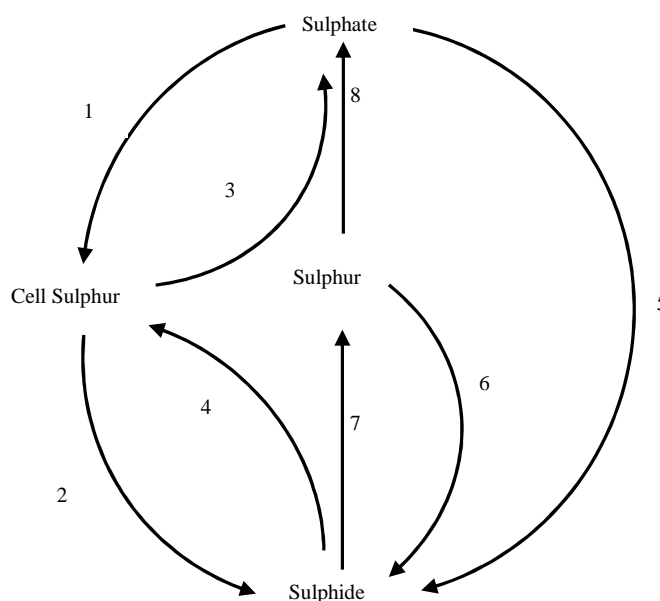


Figure 2.1 Biological sulphur cycle (Barton and Tomei, 1995)

The sulphur cycle consists of following stages: (1) assimilatory reduction of sulphate by bacteria, fungi and green plants; (2) putrefaction of dead organisms by bacteria; (3) sulphate excretion by animals; (4) sulphide assimilation by bacteria; (5) dissimilatory sulphate reduction; (6) dissimilatory elemental sulphur reduction; (7) sulphide oxidation (chemotrophic and phototrophic); (8) sulphur oxidation (chemotrophic and phototrophic).

2.1. Anaerobic Sulphate Reduction

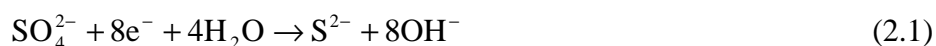
2.1.1. Microorganisms

In sulphate containing streams and acid mine drainage different genera of sulphate reducing bacteria may exist that utilise sulphate as terminal electron acceptor and an organic source as an electron donor (Moosa et al., 2000). Some common genera of SRB are *Desulfobacter*, *Desulfobulbus*, *Desulfococcus*, *Desulfotomaculum*, *Desulfomonas*, *Desulfonema*, *Desulfobacterium*, *Desulfovibrio*, *Desulfosarcina*,

Desulfoarculus, *Desulfomonile*, *Desulfobacula*, *Thermodesulforhabdus* and *Desulfacinum*.

2.1.2. Carbon and Energy Source for Sulphate Reducing Bacteria

Different types of carbon and energy sources used by the SRB for sulphate reduction are discussed in this section. Lens et al. (1998) reported that SRB are very diverse in their carbon source utilisation and the metabolic activities. The carbon and energy source provides the energy for the growth and maintenance of SRB. SRB carry out sulphate reduction based on the reaction 2.1 (Herlihy et al., 1987).



In reaction 2.1, the electrons which are needed for the sulphate reduction are generated by the oxidation of a carbon and energy source (lactate, acetate, propionate etc). A schematic representation of the sulphate reduction metabolism coupled with the utilisation of carbon source is shown in Figure 2.2. The ATP produced, using the energy released from oxidation of the carbon source, is utilised for the reduction of sulphate to sulphide. In most instances the electron donor and the carbon source are the same compound. Only when hydrogen is used as the electron donor, CO₂ is used as the carbon source. A list of carbon sources which can be utilised by the sulphate reducing bacteria is given in Table 2.1.

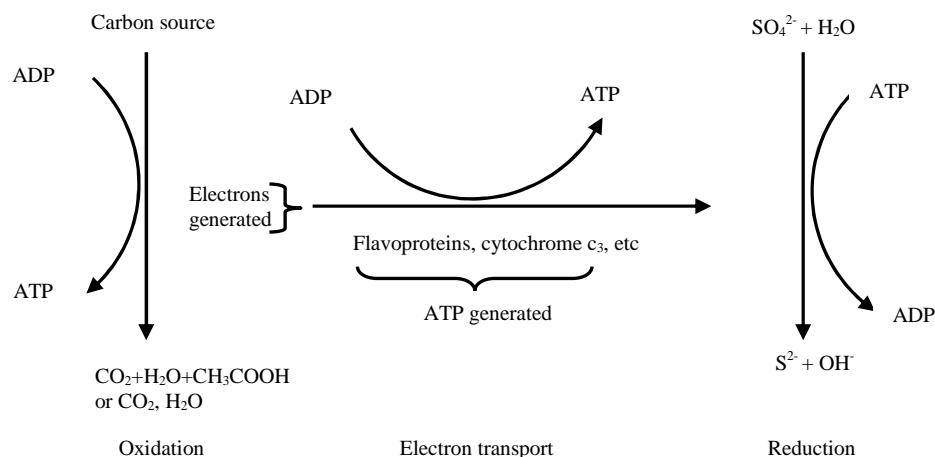


Figure 2.2 Schematic representation of sulphate reduction coupled to utilisation of an organic compound (Postgate, 1984)

Table 2.1 Electron donors and carbon sources for SRB (Hansen, 1988)

Class of compound	Carbon and energy sources
Inorganic	Hydrogen, carbon dioxide.
Monocarboxylic acids	Formate, acetate, propionate, isobutyrate, 2- and 3-methylbutyrate, higher fatty acids upto C_{20} , pyruvate, lactate.
Dicarboxylic acids	Succinate, fumarate, malate, oxalate, maleinate, glutarate, pimelate.
Alcohols	Methanol, ethanol, propanol, butanol, ethylene glycols, 1, 2- and 1, 3- propanediol, glycerol.
Amino acids	Lycine, serine, cystine, threonine, valine, leucine, isoleucine, aspartate, glutamate, phenolalanine.
Miscellaneous	Choline, furfural, oxamate, fructose, benzoate, 2-, 3-, and 4-OH-benzoate, cyclohexane carbonate, hippurate, nicotinic acid, indole, anthranilate, quinoline, phenol, p-cresol, catechol, resorcinol, hydroquinine, protocatechuate, phloroglucinol, pyrogallol, 4-OH-phenylacetate, 3-phenylpropionate, 2-aminobenzoate, dihydroxyacetone.

Desulfotomaculum thermocisternum (Nielsen et al., 1996) has been reported to utilise hydrogen as an electron donor for sulphate reduction. Some species of SRB are reported to be able to oxidise substance like alkanes, toluene, o-xylene, m-xylene, o-ethyltoluene, m-ethyl toluene, p-xylene, naphthalene, ethylbenzene and benzene (Pérez-Jiménez et al., 2001; Elshahed and McInerney, 2001; Morasch et al., 2001; Harms et al., 1999; Rueter et al., 1995; So and Young, 1999; Beller et al., 1996; Nakagawa et al., 2002). In addition alkanes (C₁₃ to C₁₈), 1-alkenes (C₁₅ and C₁₆) and 1-alkanols (C₁₅ and C₁₆) support the growth of SRB (So and Young, 1999). It was reported that *Desulfobacterium* species can degrade short-chain fatty acids, ethanol and lactate apart from acetate (Widdel, 1992). This result was confirmed with *Desulfobacterium* species found from the Guaymas basin which can degrade acetate, short-chain fatty acids, tricarboxylic acid cycle intermediates and aromatic compounds (Dhillon et al., 2003).

Sulphate reducing bacteria can be classified into two groups based on their functional ability to oxidise the organic compound completely to carbon dioxide or incompletely to acetate and CO₂. Some species of the genera *Desulfobacter*, *Desulfosarcina*, *Desulfococcus*, *Desulfobacterium*, *Desulfoorculus*, *Desulfomonile* and *Desulfonema* belong to the group of complete oxidisers. This group also encompass some SRB species like *Desulfotomaculum acetoxidans*, *Desulfotomaculum sapomandens* and *Desulfovibrio baarsii* (Postgate, 1984; Colleran et al., 1995). The SRB species that belong to incomplete oxidisers include *Desulfovibrio thermophilus*, *Desulfovibrio sapovarans*, *Desulfomas pigra*, *Thermodesulfobacterium commune* and majority of the species of genera *Desulfotomaculum*, *Desulfomonas* and *Desulfobulbus*

(Colleran et al., 1995). Table 2.2 gives the standard free energy (ΔG°) for the oxidation of different organic sources.

Table 2.2 Thermodynamic data for oxidation of different carbon and energy sources during biological sulphate reduction (Postgate, 1984)

Reaction	ΔG° (Kcal/reaction; Kcal/mole of SO_4^{2-})	Type
4H_2 (hydrogen) + $\text{SO}_4^{2-} \rightarrow 4\text{H}_2\text{O} + \text{S}^{2-}$	-29.66	Complete Oxidation
CH_3COO^- (acetate) + $\text{SO}_4^{2-} \rightarrow \text{H}_2\text{O} + \text{CO}_2 + \text{HCO}_3^- + \text{S}^{2-}$	-2.97	
4HCOO^- (formate) + $\text{SO}_4^{2-} \rightarrow 4\text{HCO}_3^- + \text{S}^{2-}$	-43.70	
$4\text{CH}_3\text{COCOO}^-$ (pyruvate) + $\text{SO}_4^{2-} \rightarrow 4\text{CH}_3\text{COO}^- + 4\text{CO}_2 + \text{S}^{2-}$	-79.20	Incomplete Oxidation
$2\text{CH}_3\text{COCOO}^-$ (lactate) + $\text{SO}_4^{2-} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{CO}_2 + 2\text{H}_2\text{O} + \text{S}^{2-}$	-33.60	
$2\text{C}_4\text{H}_4\text{O}_5^{2-}$ (malate) + $\text{SO}_4^{2-} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{CO}_2 + 2\text{HCO}_3^- + \text{S}^{2-}$	-43.30	
$2\text{C}_4\text{H}_2\text{O}_4^{2-}$ (fumarate) + $2\text{H}_2\text{O} + \text{SO}_4^{2-} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{CO}_2 + 2\text{HCO}_3^- + \text{S}^{2-}$	-45.50	
$4\text{C}_4\text{H}_4\text{O}_4^{2-}$ (succinate) + $3\text{SO}_4^{2-} \rightarrow 4\text{CH}_3\text{COO}^- + 4\text{CO}_2 + 4\text{HCO}_3^- + 3\text{S}^{2-}$	-36.00	

ΔG° : Standard free energy for the reaction.

Postgate (1984) indicated that lactate is the widely used carbon source by most SRB species. The disadvantage of using lactate is that most of the SRB species oxidise lactate partially to acetate and CO₂. For this reason a large amount of lactate is needed to reduce the sulphate which may not be a feasible option. In addition, due to the release of acetate, the chemical oxygen demand (COD) of the effluent stream increases. The incomplete oxidation of carbon sources to acetate can be attributed to the lower value of free energy for the oxidation of acetate to carbon dioxide which prevents further oxidation of acetate to carbon dioxide (Postgate, 1984).

2.1.3. Metabolism of Carbon and Energy Sources by Sulphate Reducing Bacteria

2.1.3.1. Acetate Metabolism

Acetate is one of the main organic sources that is utilised by SRB. *Desulfobacter* species utilises acetate as the sole carbon source, some species in this genera utilise hydrogen and/or ethanol (Widdel and Pfennig, 1977; Colleran et al., 1995). All incomplete oxidisers oxidise other organic sources to acetate and for this reason acetate becomes an important intermediate in the biological sulphate reduction process. *Desulfobacter* employs the cyclic citric acid cycle pathway for the oxidation of acetate. In this cycle the co-enzymeA (CoA) is transferred from the succinyl CoA to the acetyl CoA. During this process ATP is formed by the substrate level phosphorylation. As discussed earlier, in Figure 1.2, the ATP formed is utilised for the reduction of sulphate by the SRB. The uniqueness of the citric acid cycle is that it possesses the ferredoxin independent α -ketoglutarate dehydrogenase, NAD, malate synthase and the ATP-citrate

lyase. In general acetate is oxidised via a variation of the citric acid cycle (Hansen, 1988). Figure 2.3 shows the citric acid cycle for *Desulfobacter postgatei* (Colleran et al., 1994).

Species like *Desulfovibrio baarsi* and some species belonging to genera *Desulfotomaculum*, *Desulfococcus*, *Desulfosarcina* and *Desulfobacterium* are also capable of oxidising acetate completely to CO₂. It has been reported that *Desulfotomaculum* growing solely on acetate exhibits very slow growth rate (doubling time: 30 hours) when compared to *Desulfobacter* which shows a doubling time of 15-20 hours (Colleran et al., 1995). *Desulfotomaculum* cells possess a non-cyclic pathway which involves the splitting up of two carbon units into CH₃-tetrahydropterin and CO which then oxidises the two carbon units to CO₂, as shown in Figure 2.4 (Colleran et al., 1995). This process does not possess substrate level phosphorylation instead it consumes ATP and this explains its poor growth when compared to *Desulfobacter* species.

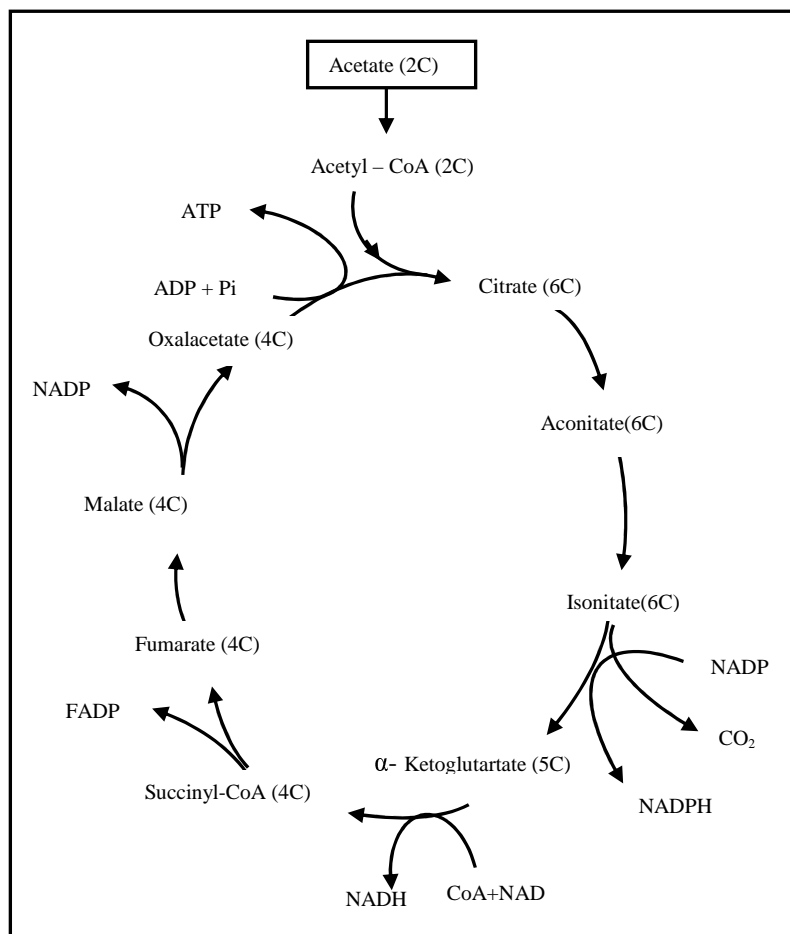


Figure 2.3 Pathway for acetate oxidation via citric acid cycle in *Desulfobacter postgatei* (Colleran et al., 1994)

NADP: nicotinamide-adenine dineucleotide phosphate; ADP: adenosine diphosphate; ATP: adenosine triphosphate; CoA: coenzyme A; FADP: flavin adenine dineucleotide phosphate; Pi: phosphate ion.

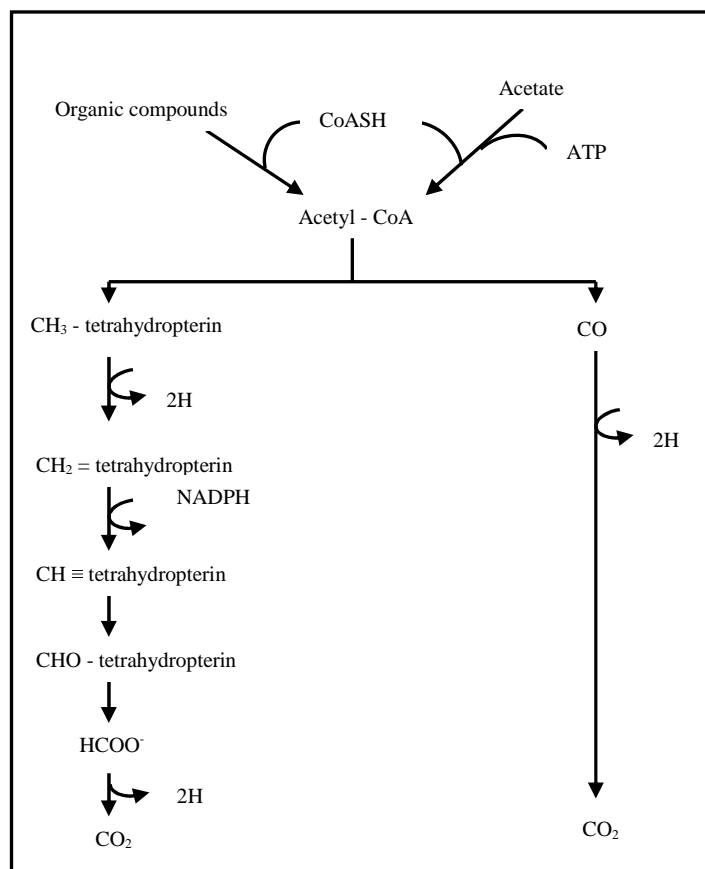


Figure 2.4 Non-cyclic carbon monoxide dehydrogenase pathway for oxidation of acetyl groups by *Desulfotomaculum acetoxidans* and other complete oxidisers growing on higher carbon compounds (Colleran et al., 1995)

2.1.3.2. Propionate Metabolism

While *Desulfobacter* species utilise acetate, the incomplete oxidizer *Desulfobulbus* species can solely grow on propionate (Gibson, 1990). The formed acetate is further converted to CO_2 by acetate utilising bacteria. Propionate, next to acetate, is an important intermediate in the biological sulphate reduction process (Colleran et al., 1995). *Desulfobulbus* oxidises propionate in a randomising pathway (Figure 2.5). This mechanism involves the transcarboxylation of propionyl CoA to methylmalonyl CoA which is followed by the isomerisation of succinyl CoA (Colleran

et al., 1995). This mechanism also involves the substrate level phosphorylation which produces ATP. Thermodynamically the oxidation of propionate via randomising pathway is favourable (Colleran et al., 1995).

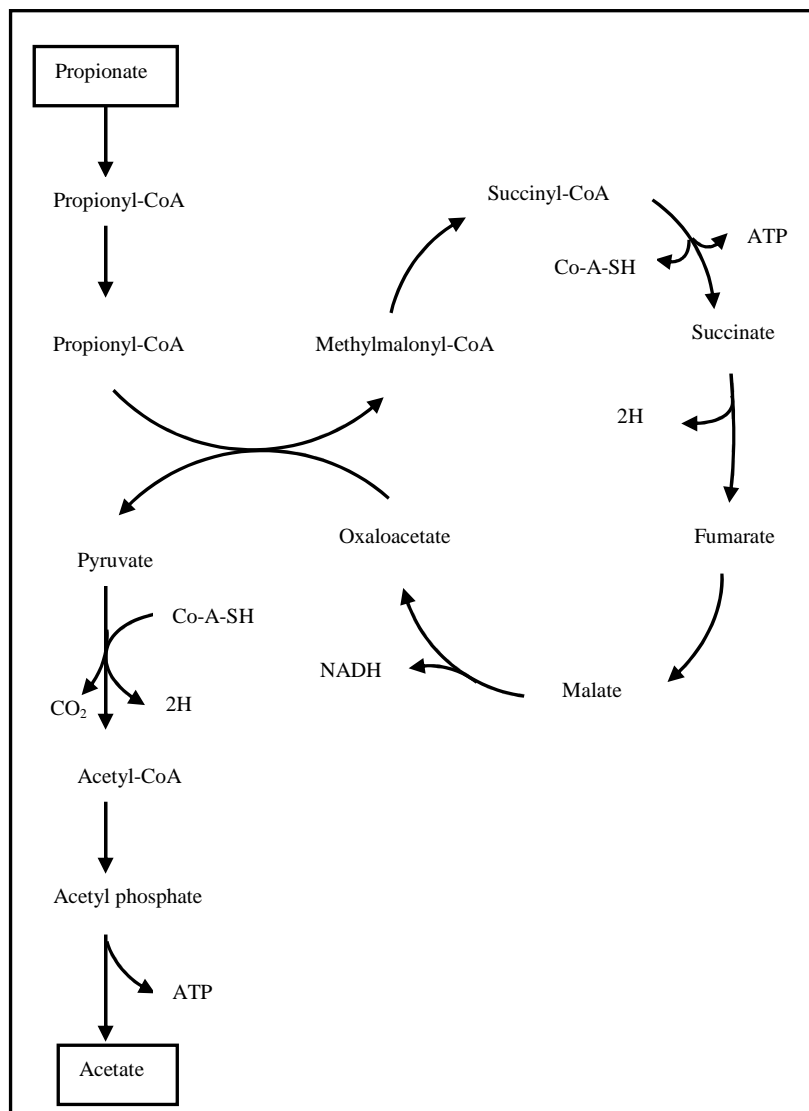
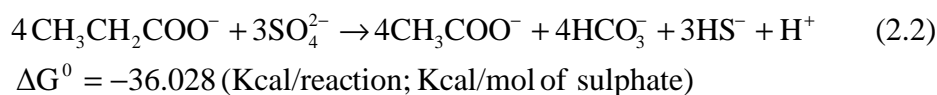


Figure 2.5 Pathway for incomplete oxidation of propionate to acetate by *Desulfobulbus propionicus* (Colleran et al., 1995)

2.1.3.3. Hydrogen Metabolism

Desulfovibrio species can use hydrogen as an energy source (Widdel, 1988). Complete oxidisers like *Desulfosarcina variabilis*, *Desulfonema limicola*, *Desulfococcus niacini* and *Desulfobacterium autotrophicum* grow slowly when compared to incomplete oxidisers like *Desulfovibrio* species. Additionally only complete oxidisers can grow autotrophically with H_2 . The *Desulfovibrio* species needs acetate in addition to hydrogen for cell synthesis (Widdel, 1988). As can be seen in Table 2.2, the free energy required for the oxidation of H_2 is -29.66 kcal which implies that when compared to acetate, H_2 is a relatively favourable energy source for the growth of SRB (Colleran et al., 1995). Two models have been proposed for the metabolism of hydrogen. The first model, shown in Figure 2.6, states that hydrogen metabolism follows a chemiosmotic cycle which involves the enzyme hydrogenase which is present in the periplasm (Peck and Legall, 1982). It was reported that most of the SRB which oxidises H_2 possesses a cytochrome (C_3) and hydrogenase. This cytochrome possesses an autooxidation mechanism which oxidises the available H_2 to protons. The eight electrons generated from this oxidation process are transferred to the inner cytoplasm where it is utilised for the reduction of sulphate. The produced protons are left in the periplasm. The hydrogen produced from the sulphate reduction is again oxidised for the production of protons and the cycle continues. In this model substrate phosphorylation for the production of ATP is absent.

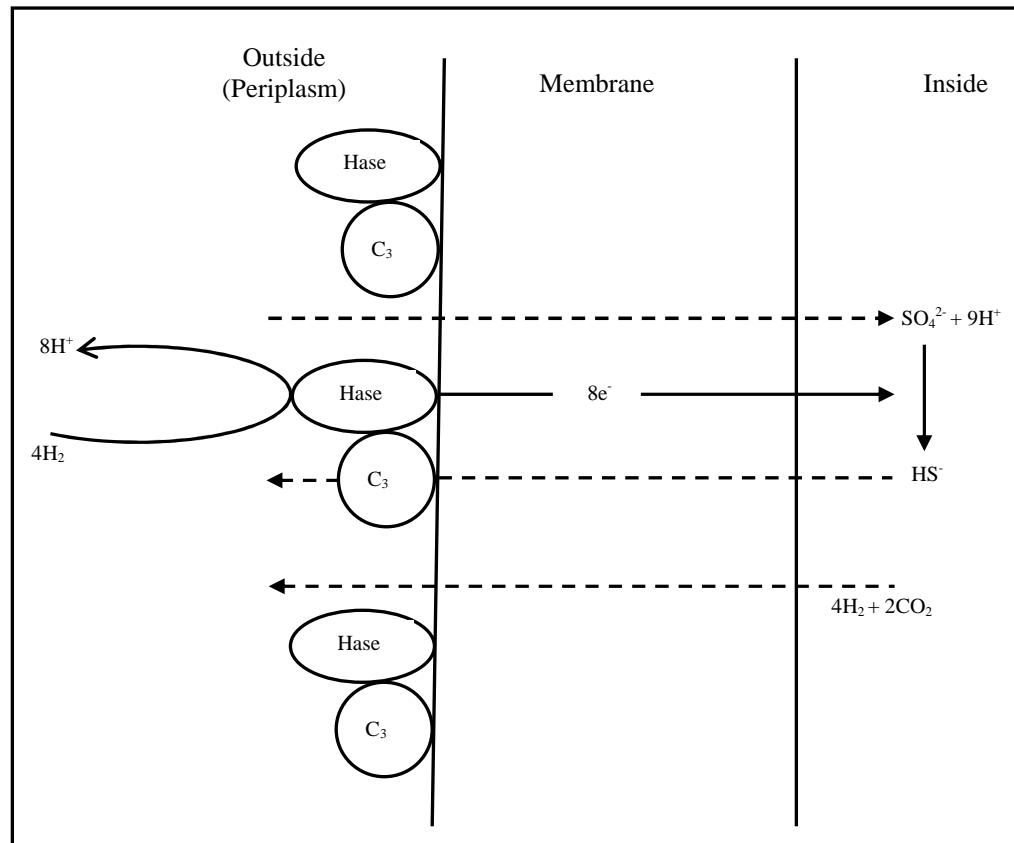


Figure 2.6 Cycling of hydrogen during sulphate reduction-Model 1 (Peck and Legall, 1982)

C₃: tetraheme cytochrome C₃; Hase: hydrogenase.

Another model proposed by Legall and Fauque (1988) is schematically illustrated in Figure 2.7. The hydrogenase present inside the periplasm controls the redox potentials of the ferridoxin and flavodoxin and thereby controlling the transport of the electrons and the protons.

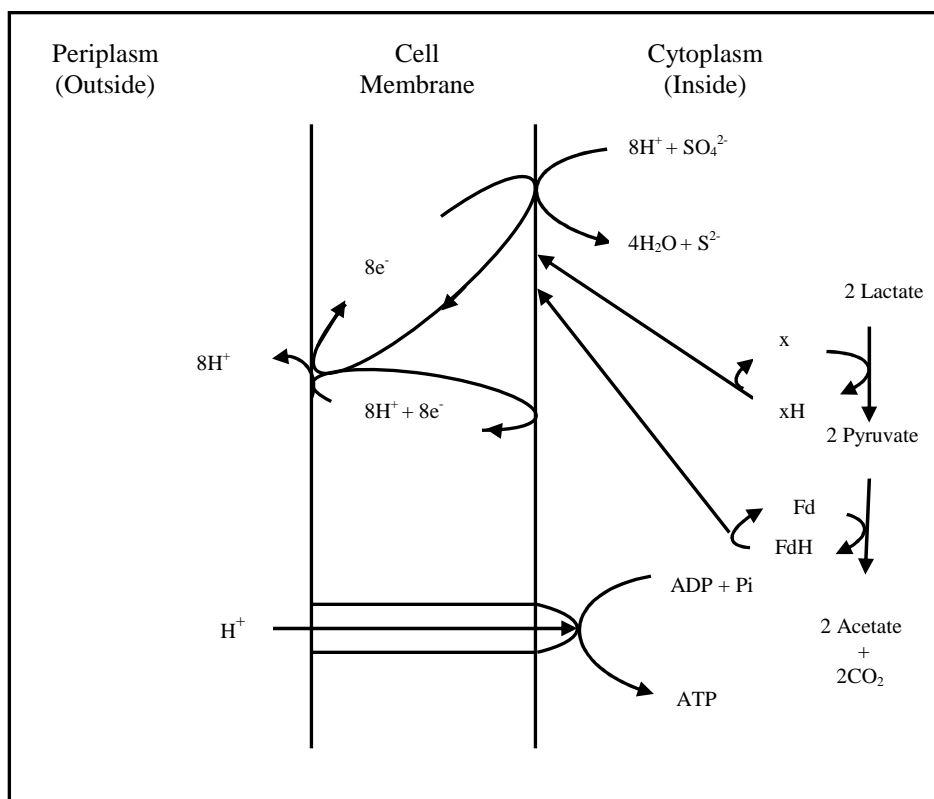


Figure 2.7 Cycling of hydrogen during sulphate reduction-Model 2 (Legall and Fauque, 1988)
Fd: ferredoxin.

2.1.4. Environmental Factors Affecting Sulphate Reducing Bacteria

Various parameters such as pH, temperature, sulphide and metal concentrations in the acid mine drainage undergoing active biological treatment will affect the growth and activity of sulphate reducing bacteria. Apart from these factors it is a well known fact that SRB are strict anaerobes. The effects of these parameters on SRB activity are discussed in the following sections.

2.1.4.1. Effect of pH on Sulphate Reducing Bacteria

One of the challenging problems in the treatment of AMD is the acidity of these streams which may adversely affect the activity of SRB. SRB strains identified are sensitive to acidic waters (Hard et al., 1997).

Pure cultures of sulphate reducing bacteria are isolated from a pond with a pH of 3.38 (Tuttle et al., 1969) and from an abandoned mine, Kam Kotia, at slightly oxidising and acidic conditions (Fortin et al., 1996). But when attempted to grow these SRB in laboratory, there was no sufficient growth below pH 5.5 in both cases. Based on the results it was concluded that SRB isolated from oxidising conditions make the environment favourable by reducing the sulphate to sulphide which will provide an alkaline environment suitable for their activity (Fortin et al., 1996). Johnson et al. (1993) reported that a species belonging to the genera *Desulfotomaculum* can grow in a environment with a pH of 2.9. Later they termed it as acid tolerant bacteria rather than acidophilic bacteria. Kolmert and Johnson (2001) reported that a mixed acidophilic SRB culture was able to grow in a medium with a pH of 3.0 and the result supports the view by Postgate (1984) that mixed SRB cultures are more tolerant to extreme conditions than pure cultures. Elliott et al. (1998) investigated the effect of acidic conditions on SRB species at pH values of 4.5, 4.0, 3.5, 3.25 and 3.0 in a porous up-flow bioreactor. SRB removed 38.3% of sulphate at pH of 3.25 and 14.4% at pH of 3.0. The reactor was operated at a flow rate of 0.6 mL/min and feed sulphate concentration of 1.0 g/L. The available information in the literature indicates that SRB in general are not acidophilic. However, acid-tolerant SRB have been identified in different works.

2.1.4.2. *Effect of Temperature on Sulphate Reducing Bacteria*

The sulphate reducing bacteria can be classified into mesophiles (growth temperature < 40°C), moderate thermophiles (growth temperature: 40-60°C) and extreme thermophiles (growth temperature > 60°C) based on their optimum growth temperature.

Pilot scale experiments confirm that sulphate reduction increases when the reaction temperature is increased from 20 to 32°C by employing a mesophilic SRB culture (van Houten et al., 1997; Weijma et al., 1999). Moosa (2000) employed a mixed culture consisting of acid producers, methane producers and sulphate reducers and conducted batch experiments. It was reported that sulphate reduction rate increased with increasing the reaction temperature from 20 to 35°C. Further increase of temperature to 40°C led to inactivity of bacteria.

Stetter et al. (1993) isolated a number of thermophilic SRB strains from the Thistle reservoir. Table 2.3 shows the growth temperature and carbon and energy sources for these strains. Some sulphate reducers such as *Desulfotomaculum* species are endospore formers, and are considered to survive in extreme environments (Widdel, 1992). The temperature and pH range for growth of thermophilic SRB species as reported by different workers are presented in Table 2.4. The suitable pH, temperature and carbon source for various genera of SRB are summarized in Table 2.5.

Table 2.3 Growth temperature for SRB isolated from Thistle reservoir (Stetter et al., 1993)

Species	Growth temperature (°C)		Laboratory substrates	Electron acceptor
	Range	Optimum		
<i>Archaeoglobus fulgidus</i>	60-94	83	Lactate or cell extracts	SO ₄ ²⁻
<i>Archaeoglobus profundus</i>	65-90	82	H ₂ /CO ₂ + cell extracts or H ₂ /CO ₂ + acetate	SO ₄ ²⁻
' <i>Achaeoglobus lithotrophicus</i> sp.nov.'	63-89	80	H ₂ /CO ₂	SO ₄ ²⁻

Table 2.4 Growth range and optimum temperature and pH for some thermophilic SRB

Species	Temperature(°C)		Spore formation	pH		Reference
	Optimum	Range		Range	Optimum	
<i>Thermodesulforhabdus norvegicus</i>	60	44-74	No	6.1-7.7	6.9	Beeder et al., 1995
<i>Desulfotomaculum luciae</i>	-	50-70	Yes	-	-	Liu et al., 1997
<i>Desulfotomaculum solfataricum</i>	60	48-65	Yes	6.4-7.9	7.3	Goorissen et al., 2003
<i>Desulfotomaculum thermobenzoicum</i> <i>subsp. thermosyntrophicum.</i>	55	45-62	Yes	6.0-8.0	7.0-7.5	Plugge et al., 2002
<i>Desulfotomaculum thermocisternum</i>	62	41-75	Yes	6.2-8.9	6.7	Nilsen et al., 1996
<i>Desulfotomaculum thermosapovorans</i>	50	35-60	Yes	-	7.2-7.5	Fardeau et al., 1995
<i>Desulfacinum infernum</i>	64	-	No	-	71.-7.5	Rees et al., 1995

Table 2.5 Morphology, carbon source, pH and temperature range for growth of some SRB species (Widdel, 1988)

Genus	Morphology and size	Carbon and energy source	pH		Temperature (°C)
			Range	Optimum	Range
<i>Desulfobacter</i>	curved (1-2µm)	Acetate	6.2-8.5	7.3	28-32
<i>Desulfobulbus</i>	tapered spheres (1-1.3µm)	propionate, lactate, pyruvate, ethanol, propanol	6.0-8.6	7.2	28-39
<i>Desulfococcus</i>	spheres, in clusters (1.5-2.2µm)	formate, acetate, lactate, butyrate, pyruvate	-	-	30-36
<i>Desulfotomaculum</i>	Straight	lactate, pyruvate, acetate, ethanol, hydrogen	-	-	-
<i>Desulfomonas</i>	-	-	6.5-8.5	7.2	30
<i>Desulfonema</i>	long filaments	acetate, malate, benzoate, pyruvate	-	-	-
<i>Desulfosarcina</i>	clusters of rod shaped cell (1-1.5 µm)	formate, acetate, butyrate, propionate	6.9-7.0	7.4	33-38
<i>Desulfovibrio</i>	curved (2.5 - 10µm)	Lactate	7.5	-	25-35

2.1.4.3. Effect of Sulphide on Sulphate Reducing Bacteria

When the sulphate is reduced to sulphide, the environment pH plays an important role in the existing form of produced sulphide. The sulphide can be present in different

forms like hydrogen sulphide in both solution and gas, HS^- and S^{2-} . The state of sulphide solely depends on the pH of the environment. At a pH of 7.0 most of the sulphide concentration is in the hydrogen sulphide form (Perry and Green, 1984). The relation between the concentrations of undissociated hydrogen sulphide in the liquid and gas phase is based on the Henrys law as shown by reaction (2.3). The value of absorption coefficient, α , at 30°C is equal to 1.99 (Lens et al., 1998).



Figure 2.8 show the influence of pH on the existing forms of hydrogen sulphide. At low pH the produced hydrogen sulphide exists in undissociated form and as the pH increases it dissociates into HS^- and S^{2-} . The dissociation process is given by reactions (2.4) and (2.5).

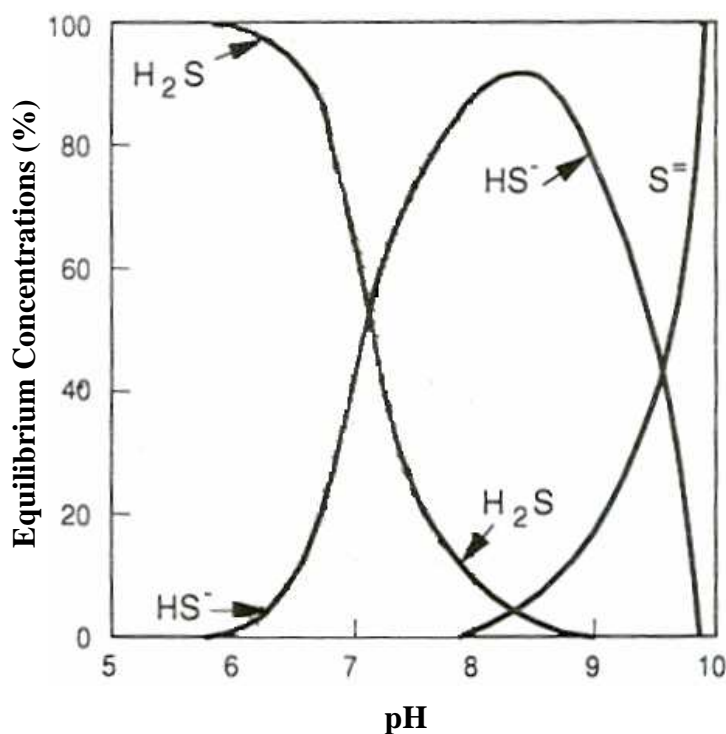


Figure 2.8 Prevalent forms of sulphide at different pH values (Lens et al., 1998)



Information available on sulphide toxicity and the mechanism of toxicity is vague. It has been reported that sulphide is absorbed into the cell and destroys the proteins thereby making the cell inactive (Postgate, 1984). If this is the case, bacteria should not be able to resume its activity once all the sulphide is removed. By contrast, it was reported that the sulphide inhibition is reversible in SRB inoculated bioreactors (Ries et al., 1992). Another theory states that the precipitation of trace element metals, as metal sulphides, which are essential for the growth of SRB, is the cause for the decreased activity (Bharathi et al., 1990). In addition to the uncertainty with respect to inhibitory mechanisms of sulphide, contradictory reports exist with respect to inhibitory effects of various forms of sulphide. Some researchers report the sulphide inhibition based on the total sulphide (Hilton and Oleszkiewicz, 1988; Cord-Ruwisch et al., 1988), and some based on the undissociated H_2S (McCartney and Oleszkiewicz, 1991; McCartney and Oleszkiewicz, 1993). Speece (1983) stated that only the undissociated H_2S is capable of entering into the cell membrane. Later it was shown that the bacteria has two threshold inhibition levels, one for the undissociated H_2S and the other for the total sulphide (O’Flaherty and Colleran, 1998) and this level depends on the environmental pH. At a pH less than 7.2, undissociated H_2S is dominant and it will reach the threshold limit. At a pH above 7.2 the total sulphide is responsible for the inhibitory effect (O’Flaherty and Colleran, 1998). It is not easy to compare the inhibitory/toxic values reported in the literature, as the inhibition has been assessed based on either growth, substrate degradation, sulphate reduction or cellular yield. Table 2.6 gives the

threshold limit for 50% inhibition in batch growth at different pH values for various strains of SRB (O’Flaherty and Colleran, 1998). As can be seen, the sulphate reducing bacteria are less sensitive to total sulphide when the pH is increased from 6.8 to 8.0 and more sensitive to the undissociated sulphide concentration. In addition, as the pH increases less concentration of undissociated H₂S is needed to inhibit the growth by 50%.

Table 2.6 pH dependency of sulphide inhibition for some SRB strains (O’Flaherty and Colleran, 1998)

Bacterial group	Substrate	IC50 value (mg/L)							
		pH = 6.8		pH=7.2		pH=7.6		pH=8.0	
		TDS	H ₂ S	TDS	H ₂ S	TDS	H ₂ S	TDS	H ₂ S
<i>Desulfotomaculum magnum</i>	acetate	443	239	671	218	660	105.6	659	46
<i>D.acetoxidans</i>	acetate	487	263	775	252	1360	218	1500	105
<i>D.vulgaris</i>	H ₂ /CO ₂	554	299	840	273	1343	215	1499	105
<i>D.sapovorans</i>	butyrate	513	277	796	259	1133	181	1170	82
<i>D.postgatei</i>	acetate	583	315	926	301	1248	200	1119	78
<i>D.multivorans</i>	ethanol	498	269	851	277	1383	221	1488	105
<i>D.propionicus</i>	propionate	223	120.5	355	115	500	80	525	37
Mixed SRB adapted to sulphate	acetate	374	202	550	179	867	139	990	69
	H ₂ /CO ₂	505	273	760	247	1127	180	1243	87
	propionate	328	177	410	134	595	95.2	572	40
	butyrate	593	320	900	292.5	1875	300	2005	140
	ethanol	561	303	880	286	878	140.5	1130	79

TDS: Total dissolved sulphide; H₂S: Undissociated hydrogen sulphide. IC50: Concentration which reduces growth rate by 50%.

The noncompetitive inhibition model has been used by different workers to describe the effects of total sulphide and undissociated H₂S (Maillacheruvu and Parkin

1996; Kaksonen et al., 2004). The sulphide inhibition constants as determined by these workers are given in Table 2.7. A previous report indicated that there is a difference in activity of free cells and biofilms employed for sulphate reduction (Kaksonen et al., 2004). It is also said that an extracellular polymeric substance that binds the biofilms protects the cells from the toxic effects to some extent (Teitzel and Parsek, 2003).

Table 2.7 Inhibition constants (K_i) for sulphide toxicity on substrate utilisation activity

Bacterial group	Substrate	Reactor and mode	Temperature (°C)	pH	K_i , mg/L		Reference
					TDS	H ₂ S	
Mixed SRB culture	acetate	Serum bottle, B	-	-	35.0	8.0	Maillacheruvu and Parkin, 1996
Mixed SRB culture	acetate	FBR, B	35.0	6.9-7.3	356.0	124.0	Kaksonen et al., 2004

TDS: Total dissolved sulphide; H₂S: Undissociated hydrogen sulphide; FBR: Fluidized bed reactor; B: batch.

As can be seen in Table 2.7, the inhibition constants for the biofilms is significantly higher than that for the free cells which substantiates the statement by Teitzel and Parsek (2003). As stated earlier the inhibition of sulphide on growth, sulphate reduction, substrate degradation and cellular yield has been reported by various workers. Table 2.8 summarizes the inhibitory levels of sulphide from different works.

Table 2.8 Inhibitory sulphide concentration reported in different works

Bacterial group	Substrate	Reactor type and mode	Temperature (°C)	pH	TDS (mg/L)	H ₂ S (mg/L)	Inhibition	Reference
UASB sludge	Ethanol	UASB	35	7.1-8.0	1700-9951	570-610	100% ¹	Kalyuzhnyi et al., 1997
UASB sludge	Acetate	batch reactor	30	7.0	521	217	50% ²	Visser et al., 1996
UASB sludge	Acetate	serum bottle	35	7.2-7.4	615	161	50% ²	Visser et al., 1996
UASB sludge	Acetate	serum bottle	35	7.0	699	270	50% ¹	Yamaguchi et al., 1999
<i>Desulfovibrio desulfuricans</i>	Lactate	serum bottle, B	35	7.2-7.6	82.3	-	50% ¹	McCartney and Oleszkiewicz, 1991
Mixed SRB (suspended sludge)	Lactate	STR, B	35	7.1-7.3	-	300	50% ¹	McCartney and Oleszkiewicz, 1993
				8.0	-	185	50% ¹	

¹Inhibition of sulphate reduction activity; ²Inhibition of growth; ³Inhibition of cellular yield; ⁴Inhibition of substrate utilisation; UASB: up-flow anaerobic sludge bed reactor; STR: stirred tank reactor; B: batch; C: continuous; TDS: Total dissolved sulphide; H₂S: Undissociated hydrogen sulphide.

Table 2.8 (Contd.) Inhibitory sulphide concentrations reported in different works

Bacterial group	Substrate	Reactor type and mode	Temperature (°C)	pH	TDS (mg/L)	H ₂ S (mg/L)	Inhibition	Reference
<i>Desulfovibrio desulfuricans</i>	lactate	STR, C	35	7.0	600	-	95% ³ + 69% ⁴	Okabe et al., 1992
<i>Desulfovibrio desulfuricans</i>	lactate	STR, B	35	7.0	440	-	70% ³	
Mixed SRB (suspended sludge)	lactate	STR, B	35	7.2-7.6	-	80	50% ¹	

¹Inhibition of sulphate reduction activity; ²Inhibition of growth; ³Inhibition of cellular yield; ⁴Inhibition of substrate utilisation; UASB: up-flow anaerobic sludge bed reactor; STR: stirred tank reactor; B: batch; C: continuous; TDS: Total dissolved sulphide; H₂S: Undissociated hydrogen sulphide.

2.1.4.4. Effect of Metals on Sulphate Reducing Bacteria

In general, AMD incorporates heavy metals such as iron, zinc, copper, manganese and lead at high concentrations which may be toxic or inhibitory to the activity of SRB. Steps are taken to prevent the toxic effects of metals on SRB by finding new strains of metal tolerating bacteria and by employing bioreactors with special designs. Utgikar et al. (2002) reported that the inhibition kinetic constants (based on rate of sulphate reduction) for Cu and Zn as 17.9 ± 2.5 and $25.2 \pm 1.0 \text{ mM}^{-1}$, respectively and the toxic kinetic constants (based on microbial growth) for Cu and Zn as 10.6 and 2.9 mM^{-1} . Another study reported that a strain of SRB (UFZ B 407) was able to tolerate the presence of aluminium, lead, uranium, iron, chromium, copper, silver, nickel, manganese and cobalt at maximum concentrations of 50 mM, 10 mM, 0.01 mM, 50 mM, 30 mM, 10 mM, 100 mM, 10 mM, 50 mM and 10 mM, respectively (Hard et al., 1996). In addition, strains of metal tolerating sulphate reducing bacteria were isolated and when tested, displayed activity in the stream containing 100 ppm of Cu and 30 ppm of Fe (Garcia et al., 2001).

Contrary to common belief that only soluble metallic ions can be toxic or inhibitory, Utgikar et al., 2001 demonstrated that insoluble metal compounds could also affect the activity of SRB. Utgikar et al. (2001) reported that the insoluble metal sulphide formed is not toxic to the SRB by itself but it blocks the access to substrate and the nutrients that are essential for bacteria by forming a precipitate coating the SRB. To reduce the inhibitory effects of metals and to increase the pH of AMD, a part of the treated AMD can be recycled and mixed with the influent AMD (Glombitza, 2000). The sulphide present in the treated AMD will react with the present metals and precipitate. In

another study an extractive silicone membrane module was accommodated with the original bioreactor setup (Chuichulcherm et al., 2001). The treated AMD from the bioreactor was passed to the outer part of the membrane (which was selectively permeable for H_2S) while the untreated AMD was pumped through the inner part of the membrane. The H_2S permeates from the outer side through the membrane and reacts with metal ions present in the inner part to form metal sulphides. The metal sulphides were removed as fine suspensions and the AMD without metal ions was fed to the bioreactor.

2.1.5. Reactors Employed for Anaerobic Sulphate Reduction

A variety of reactor configurations such as up-flow anaerobic sludge bed reactors (Colleran et al., 1994; Sanchez et al., 1997), stirred tank reactors (Moosa et al., 2002 and 2005; Herrera et al., 1997) and packed bed reactors (Jong and Parry, 2003; Chang et al., 2000) have been used to study anaerobic reduction of sulphate and to treat acid mine drainage. Biological sulphate reduction can be achieved with freely suspended bacterial cells or immobilised cells. Application of freely suspended cells in continuous bioreactors dictates a high residence time to prevent cell washout. In other words a continuous reactor with freely suspended cells has to be operated at low flow rate and high residence time. In an immobilized cell bioreactor the biomass residence time becomes uncoupled from the hydraulic residence time; therefore it is possible to operate the reactor at high flow rate without cell washout. The biofilm formed in the immobilised cell bioreactors also offers more resistance to extreme conditions such as low pH, high metal concentrations etc.

Kolmert and Johnson (2001) investigated the tolerance of mixed SRB culture to acidic environment in an up-flow packed bed bioreactor, using porous glass beads as a carrier matrix. The average volumetric reduction rates of 0.010 to 0.013 g/L-day were achieved in bioreactors containing mixed culture of acidophilic and neutrophilic SRB with a feed pH of 4.0. Kolmert and Johnson reported that sulphate reduction occurred at a pH of 3.0 but with a lower rate.

Jong and Parry (2003) used an up-flow packed bed bioreactor with sand as carrier matrix for anaerobic reduction of sulphate with mixed culture of sulphate reducing bacteria. Feed contained 2.5 g/L sulphate and 10 mg/L of each Al, As, Cu, Zn, Ni and Fe metals. The highest volumetric reduction rate of 0.019 g/L-h, was observed at a volumetric loading rate of 0.155 g/L-h at 25°C.

Chang et al. (2000) demonstrated that solid waste materials including oak chips (OC), spent oak from shiitake mushroom farms (SOS), spent mushroom compost (SMC), sludge from a wastepaper recycling plant (SWP) and organic-rich soil (ORS) can be used as electron donors and immobilisation matrices to treat acid mine drainage. The bioreactors were inoculated with an anaerobic digester fluid. The feed sulphate concentration was 2.58 g/L and total dissolved metal concentrations were 500 mg/L iron, 100 mg/L zinc, 50 mg/L manganese and 50 mg/L copper. Temperature was maintained at 25°C and the pH of the medium was adjusted to 6.8. At a volumetric loading rate of 0.005 g/L-h, the highest volumetric reduction rate of 0.005 g/L-h was achieved in the bioreactor packed with sludge from wastepaper recycling plant.

Elliott et al. (1998) conducted experiments in a packed bed bioreactor to investigate the effect of pH on the anaerobic sulphate reduction. The column was packed with sand and the pore volume was 783 mL. In this study Postgate Medium B (1979)

without iron sulphate was pumped through the column at a rate of 0.6 mL/min. The bioreactor was operated at a given pH until a steady state was reached. After attaining the steady state, the pH of the feed was lowered step by step. Initially the pH of the feed was adjusted to 4.5 and then it was decreased to 4.0, 3.5, 3.25 and 3.0 under continuous flow conditions. The bioreactor removed 45.1%, 44.6%, 35.5%, 38.3% and 14.4% of initial sulphate at pH 4.5, 4.0, 3.5, 3.25 and 3.0, respectively.

It has always been challenging to use CO₂ and H₂ as the carbon and energy source for sulphate reduction in a packed-bed bioreactor. Foucher et al. (2001) successfully proved that CO₂ and H₂ can be used to treat Chessy mine drainage in an up-flow packed bed bioreactor with a special packing to provide good mass transfer between hydrogen and liquid. The pH of the feed was 2.55 and the sulphate concentration was 5.8 g/L and metals like Fe²⁺, Fe³⁺, Zn, Cu, Al, Mn, Co, Ni and Pb were present in concentrations of 1470, 70, 320, 160, 210, 5.5, 0.06, 0.4 and 0.5 mg/L, respectively. Although the feed sulphate concentration was 5.8 g/L, a part of the effluent stream was recycled and the concentration of sulphate in the inlet stream was reduced to 0.6 to 0.8 g/L. The maximum flow rate employed was 900 mL/h (residence time of 0.9 days), and the corresponding volumetric reduction rate achieved was 0.2 g/L-h.

Chen et al. (1994) studied the kinetics and stoichiometry of sulphide formation in a packed-bed bioreactor using sea sand as carrier matrix. Lactate was used as a carbon source and the SRB species *Desulfovibrio desulfuricans* was used as an inoculum. At the volumetric loading rate of 0.138 g/L-h the maximum volumetric reduction rate achieved was 0.015 g/L-h.

Waybrant et al. (2002) investigated the effect of packing reactive mixtures which were basically waste products. Two up-flow packed-bed bioreactor containing two

different reactive mixtures were used: first one containing leaf mulch, sawdust, sewage sludge, and wood chips and the second containing leaf mulch and sawdust. The maximum volumetric reduction rates achieved in the first and second columns were 0.003 and 0.005 g/L-h, respectively.

Lin and Lee (2001) studied anaerobic sulphate reduction in a fixed bed biofilm column bioreactor. The Plastic Ballast rings were chosen as the supporting media for bio-film formation. The feed sulphate concentration was 0.9 g/L. The reactor volume was 42.65 L, which yields a hydraulic residence time of 2.5 days. The reactor temperature was controlled at 35°C. The conversion achieved was 98%.

The specification of bioreactors, operating conditions and the kinetic data as reported by various works are summarised and compared with the results of present work in Tables 5.5 and 5.6, included in results section of this thesis.

3. RESEARCH OBJECTIVES AND EXPERIMENTAL PROGRAMME

Anaerobic reduction of sulphate can be achieved with freely suspended cells in a continuous stirred tank bioreactor but the bioreactor has to be run at high residence times to prevent the cell washout. Application of long residence times results in an increased bioreactor size. In an immobilized cell system the biomass residence time becomes uncoupled from the hydraulic residence time. Thus, it is possible to operate the bioreactor at short residence times, while maintaining a high biomass concentration which leads to faster reaction rates. Information regarding the kinetics of anaerobic sulphate reduction in biofilm systems is limited in the existing literature. The objectives of this work were thus:

- To compare the performance of the packed-bed bioreactors with three immobilisation matrices, namely sand, foam biomass support particle (foam BSP) and glass beads and to verify the most suitable carrier for immobilization of the cells.
- To study the effects of initial sulphate concentration and its volumetric loading rates on the kinetics of the sulphate reduction in immobilized cell bioreactors.

3.1. Phase 1

Firstly, three immobilisation matrices namely sand, foam BSP and glass beads were selected. Three continuous experimental runs were thus conducted using three

identical up-flow packed bed bioreactors, each packed with one of the carrier matrices. The initial feed sulphate concentration was maintained constant at 1.0 g/L for all the three experimental runs. The pH of the feed was adjusted to 7.0 and the experiments were conducted at room temperature (22°C).

3.2. Phase 2

The second phase of experiments was performed to investigate the effect of initial sulphate concentration and its volumetric loading on the kinetics of anaerobic sulphate reduction. Three continuous experimental runs were carried out with initial feed sulphate concentrations of 1.0, 2.5 and 5.0 g/L. Sand was used as a carrier matrix for all the three runs since the performance of the bioreactor with sand was superior as compared to foam BSP and glass beads. These continuous experiments were performed at room temperature (22°C) and the medium pH was maintained constant at 7.

4. MATERIALS AND METHODS

In this section the bacterial culture, medium composition, subculturing procedures, as well specification of the experimental set-ups and the procedures for the continuous experiments are discussed. Lastly the analytical techniques used to monitor influential parameters are described.

4.1. Microbial Culture and Medium

A mixed culture of sulphate reducing bacteria was enriched from the produced water of the Coleville oil field in Saskatchewan, Canada and used as an inoculum. This enrichment has been shown previously to be dominated by *Desulfovibrio* species, with heterotrophic bacteria being the minor component of the population (Nemati et al. 2001). The produced water was provided kindly by Professor G.Voordouw, Department of Biological Sciences, University of Calgary.

4.1.1. Medium

Modified Coleville Synthetic Brine (m-CSB) was used for the growth and maintenance of SRB. Sodium lactate was used as a carbon source. Modified CSB contained per L: 7 g NaCl, 0.027 g KH₂PO₄, 0.02 g NH₄Cl, 0.24 g CaCl₂.2H₂O, 0.975 g

MgSO₄·7H₂O, 1.075 g (NH₄)₂SO₄, 1.9 g NaHCO₃, 5.5 g Na-lactate (60% v/v; equivalent to 2.67 g lactate ion) and 0.5 ml trace element solution (Nemati et al., 2001). The trace element solution contained per litre: 0.5 mL concentrated H₂SO₄, 2.28 g MnSO₄·H₂O, 0.5 g ZnSO₄·7H₂O, 0.5 g H₃BO₃, 0.025 g CuSO₄·5H₂O, 0.025 g Na₂MoO₄·2H₂O, 0.045 g CoCl₂·6H₂O and 0.58 g FeCl₃. All medium components were dissolved using distilled water and the pH was adjusted to 7.0, using 2M HCl. The sulphate and lactate ion concentrations were 1.0 and 2.67 g/L, respectively.

4.1.2. Culture Conditions

Modified-CSB was prepared and dispensed in 100 mL aliquots in 120 mL serum bottles. Following the addition of media, all the bottles were flushed with nitrogen gas for five minutes. The bottles were then sealed and autoclaved for 30 minutes at 121°C. Following sterilisation the bottles were allowed to reach the room temperature. These were then used for enrichment of SRB culture and maintenance of the cultures. Initially, Coleville produced water was used as an inoculum (10% v/v). The enriched SRB culture which obtained was then used as an inoculum in subsequent subculturing and also for inoculation of bioreactors. The cultures were maintained at room temperature (22°C).

4.1.3. Medium for Bioreactor

The m-CSB used for maintenance and growth of SRB was used in the bioreactor experiments. Modified-CSB was prepared in a two (or four) litre flask, autoclaved for 30 minutes at 121°C, cooled to room temperature and then purged with nitrogen for

about 2 hours before using it as a feed for the bioreactor. To maintain anaerobic conditions, to decrease the redox potential of the medium and to prevent the contamination of medium, nitrogen was purged constantly through the medium bottle during the experiment.

4.2. Specifications of the Up-Flow Packed Bed Bioreactors

The up-flow, packed-bed bioreactors used in this study were made of glass columns (D: 4 cm and H: 36 cm) with three sampling ports at 12.5 cm intervals. A polymeric mesh pad was placed at the bottom of each bioreactor to maintain the carrier matrix. All the three sampling ports were sealed using rubber septum. The bioreactor was then packed with the designated packing material (either sand, foam BSP or glass beads) to provide a matrix for establishment of biofilm. To remove air from the bioreactor, nitrogen gas was introduced continuously to the bottom of the column at a low flow rate, prior to and during packing of the carrier matrix. Following the packing with carrier matrix, the bioreactor was sterilised in an autoclave for 30 minutes at 121°C. The glass joint at the top of the bioreactor was sealed with silicon sealant to make sure that there were no leaks from the bioreactor. Tygon tubing was used to transfer the medium into the bottom of bioreactor and to remove the effluent from the top of the bioreactor. A schematic diagram of the experimental set-up is shown in Figure 4.1.

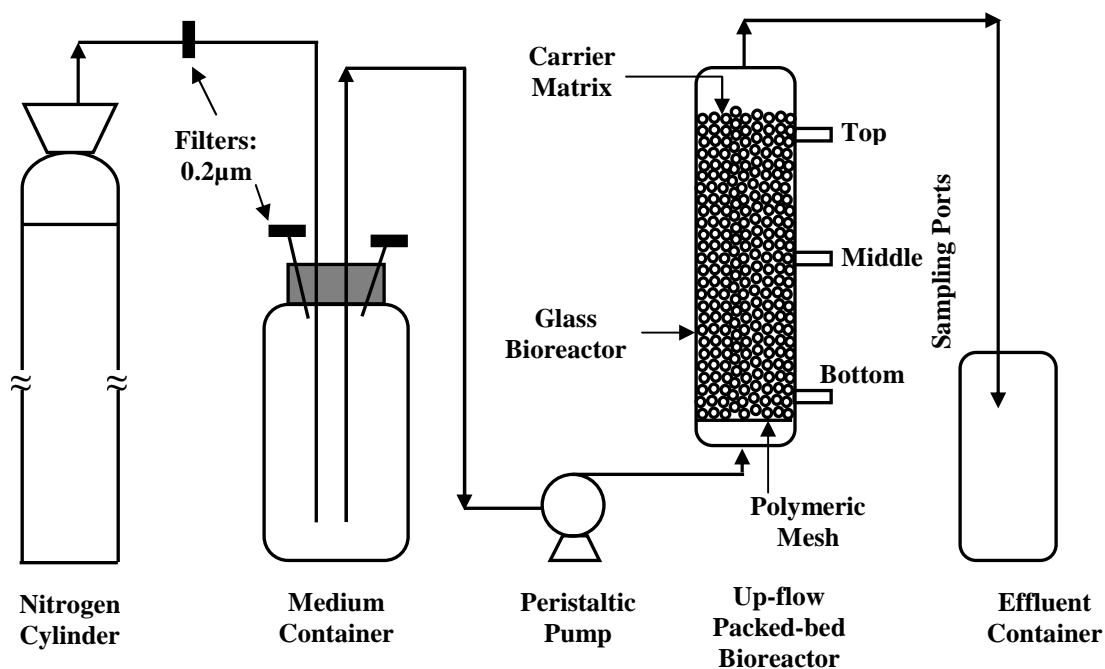


Figure 4.1 Schematic diagram of the experimental setup

4.3. Experimental Procedures

4.3.1. Batch Operation of Bioreactor

Liquid medium with the designated sulphate concentration was introduced into the bottom of the bioreactor via Tygon tubing using a peristaltic pump. Before turning the pump on, all of the tubing was washed with 70% ethanol solution to minimise the possibility of contamination. About two pore volumes of medium were pumped through the bioreactor to ensure that the medium completely filled the voids. The pump was then switched off and the bioreactor was inoculated by injecting 10 mL of SRB enrichment into each sampling port. The inoculation was performed from the bottom port to the top port to prevent the outflow of inoculum through the effluent tubing at the top. Following

the inoculation the tubing at the top and bottom of the bioreactor were clamped to keep the bioreactor free from contamination. Initially the bioreactor was operated batch-wise, during which microbial activity was monitored by determining the concentrations of sulphide and sulphate. The batch operation continued until 100% conversion of sulphate to sulphide was obtained or until the steady state is achieved (Duration of batch operation for various bioreactors were: 7, 8 and 7 days for the bioreactors fed with medium containing 1.0 g/L of sulphate and packed with foam BSP, glass bead and sand, respectively. Bioreactors fed with medium containing 2.5 and 5.0 g/L of sulphate were operated in the batch mode for 14 and 31 days, respectively).

4.3.2. Continuous Operation of Bioreactor

Once complete sulphate reduction was achieved, the bioreactor was switched to continuous mode by pumping medium into the bioreactors at a low flow rate (0.25 - 0.50 mL/h) to allow passive immobilization of the bacterial cells and formation of the biofilm. The flow rate was increased stepwise, following the establishment of steady state at a given flow rate. Steady state conditions were assumed to be established when sulphate conversion varied by less than 10 % during a period of operation equal to at least two to three residence times or when 100% conversion was observed in all three sampling ports. The stepwise increase in flow rate of the feed continued until a decrease in volumetric reduction rate of sulphate occurred. Flow rate of the feed was determined by measuring the volume of effluent collected over a certain period of time. Bioreactors were maintained at room temperature (22°C) and the feed pH was adjusted to 7.0.

Samples were taken from each port on a daily basis and analysed twice for sulphate. Because of its unstable nature, sulphide concentration was determined once. In

addition to sulphide and sulphate concentrations, pH of the effluent was also determined. The protein and organic acids concentrations (lactate and acetate ions) were determined for each regions of bioreactor after establishment of steady state conditions at each flow rate. In case of organic acids, the collected samples were stored at -75°C and analyzed at the end of each experimental run, using the HPLC. Each sample was tested three times.

The protein concentration in the liquid phase was determined only for the bioreactors operating with sand as carrier matrix, with feed sulphate concentrations of 1.0, 2.5 and 5.0 g/L. Every sample was analysed twice for protein concentration in the liquid phase.

The mean values of the steady state data (sulphate, sulphide, lactate, acetate and protein concentrations) for the corresponding volumetric loading rates were used to determine the kinetics of reaction and to compare the performance of the bioreactors at different conditions. The mean and the error involved in the steady state data for 95% confidence level were calculated, using Excel software.

4.3.4. Effects of Carrier Matrix

The effect of immobilization carrier matrix on the performance of the bioreactor (as assessed by volumetric reduction rate of sulphate) was studied by conducting three independent experimental runs, using glass beads, foam biomass support particles (foam BSP) and sand as carrier matrices (Figure 4.2). White quartz sand particles had a mesh size of -50 to +70 (average diameter: 225 µm). Foam BSP particles were made by cutting a porous scouring pad sheet, made of synthetic fibre, into cubic particles with approximate dimensions of 1×1×0.5 cm. The spherical glass beads used in this study had

a diameter of 3mm. The Brunauer-Emmett-Teller (BET) surface area of each carrier matrix was determined prior to use in the experiments. The void volume of the bioreactor packed with a designated carrier matrix was determined by filling the bioreactor with medium and measuring the volume of the liquid drained from the bottom of the bioreactor over a period of five hours. The m-CSB medium containing 1.0 g/L of sulphate ion and 2.7 g/L of lactate ion was used in all experimental runs. The concentration of lactate was around 45% higher than the theoretical value required for reduction of sulphate to ensure that the lactate was not a limiting substrate.



a



b



c

Figure 4.2 Photographs of the immobilisation matrices used in this study
a. Sand; b. Foam BSP; c. Glass beads.

4.3.3. Effects of Initial Sulphate Concentration and Volumetric Loading Rate

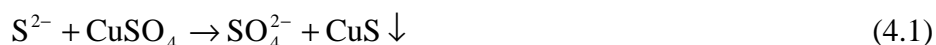
The effects of initial feed sulphate concentration and its volumetric loading rate on the kinetics of sulphate reduction were studied by conducting two additional experimental runs in bioreactors packed with the sand, fed with m-CSB media containing either 2.5 and 6.7 g/L or 5.0 and 13.3 g/L of sulphate and lactate ions, respectively. To ensure that the organic carbon source was not the limiting substrate, lactate was provided at a concentration which was around 45% higher than the theoretical value required for reduction of sulphate. Concentrations of other nutrients and trace elements were kept unchanged.

4.4. Analytical Methods

The following sections describe the analytical procedures used to monitor the concentrations of sulphide, sulphate, protein, lactate, acetate, as well as pH. The procedures for scanning electron microscopy and measurements of surface area of various carrier matrices are also described in this section.

4.4.1. Measurement of Sulphide

The concentration of sulphide was determined, using a spectrophotometric method (Cord-Ruwisch, 1985). When copper sulphate is added to a solution containing sulphide, copper sulphide precipitates according to reaction 4.1. The absorbance of the resulting mixture can be measured at 480 nm and it is proportional to sulphide concentration.



To 0.9 mL of 5.0 mM acidic copper sulphate solution, 0.1mL of a standard sodium sulphide solution was added. The acidic copper sulphate solution contained per litre: 0.8 g of copper sulphate and 4.1 mL of HCl (36.5-38%). The absorbance of the resulting solution was measured at 480 nm using SHIMADZU UVmini-1240 spectrophotometer. Using the absorbance of the standard solutions of different concentrations a calibration curve was prepared (Figure A.1, Appendix A). Concentration of sulphide in the samples taken from the bioreactors was determined using a similar procedure and the prepared calibration curve.

4.4.2. Measurement of Sulphate

The concentration of sulphate was measured using a turbidimetric method (APHA, 1992). When barium chloride is added to a solution containing sulphate, barium sulphate precipitates, according to reaction 4.2.



To 0.9 mL of a conditioning agent (0.85 mL glycerol, 0.5 mL concentrated HCl, 1.3 g NaCl, 17 mL ethanol and 1 L of distilled water), 0.1 mL of a centrifuged standard sample was added. An excess amount of barium chloride powder was then added to this mixture. The solution was mixed on a vortex mixer for one minute and kept aside for 45 minutes at room temperature to allow the precipitation of barium sulphate. The absorbance of the solution was then measured at 420 nm using SHIMADZU UVmini-1240 spectrophotometer. A calibration curve was prepared using standard solutions of sulphate (Figure A.2, Appendix A). Concentration of sulphate in the samples taken from the bioreactors was determined using a similar procedure and the prepared calibration curve.

4.4.3. Measurement of Total Protein in the Liquid Phase

Biomass concentration in liquid phase was monitored by measuring the concentration of total protein present in the liquid phase. Firstly, standard solutions of 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L of bovine serum albumin (BSA) were prepared. To 0.2 mL of the bio-rad protein assay reagent, 0.8 mL of standard BSA solution was added and the mixture was agitated by vortex mixer for about 5 seconds. The solution was kept aside for at least 5 minutes and then the absorbance of the solution was measured at 595

nm using SHIMADZU UVmini-1240 spectrophotometer. A calibration curve was prepared (Figure A.3, Appendix A). To measure the concentration of protein present in the samples from the bioreactor, the sample was sonicated for two minutes using a BRANSON Model 450 Sonifier with its output power set at 10 watts. This resulted in the release of proteins from the bacterial cells. After sonification the sample was mixed with the bio-rad protein assay reagent. Apart from sonification, the remaining steps were similar to those described already. The prepared calibration curve was used to determine the concentration of protein in the samples.

4.4.4. Measurement of Organic Acids

High performance liquid chromatography (HPLC) was used to measure the concentration of lactate and acetate ions present in the bioreactor samples. Concentrations of acetate and lactate were determined, using an 1100 Agilent Technologies HPLC, with a Zorbax SB-Aq column (4.6×150 mm packed with 5 micron particles). The mobile phase was a 10mM phosphate buffer with 1% acetonitrile. The thermostat in the HPLC was set at 30°C and the pH of the mobile phase was 2.9. The wavelength for diode array detector was set at 210 nm. Standard solutions of known concentrations of acetate and lactate were made and the calibration curve was prepared using the HPLC. The concentrations of lactate and acetate in the bioreactor samples were determined using the calibration curves provided for each compound.

4.4.5. pH Measurement

pH measurements were carried out using a Thermo Orion PerpHecT Meter (Model 330). The pH meter was calibrated using, buffer solutions (4 and 7) regularly. All the measurements were carried out at room temperature.

4.4.6. Measurement of the Carrier Matrices Surface Area

Surface area of the carrier matrices (sand, foam BSP and glass beads) were measured, using a MICROMERITICS, USA, ASAP 2000 surface area analyser. The sample was approximately weighed (sand: 1.99 g, foam BSP: 0.216 g, glass beads: 9.276 g) in a glass sample tube and degassed under vacuum at 100°C. The sample was then placed on the analysis side of the apparatus and analysed for Brunauer-Emmett-Teller (BET) surface area. Adsorption characterisation of the carrier matrices were determined by nitrogen adsorption at -196°C.

4.5.7. Scanning Electron Microscopy

The biofilm formed on the surface of the foam BSP particles was examined by scanning electron microscopy (SEM). Foam BSP particles were removed from the lower and middle parts of the bioreactor packed with foam BSP after the completion of the run and was frozen by dipping into liquid nitrogen. These were then dried at 60°C for 30 minutes to remove the moisture from the foam BSP particle. The JEOL 840A scanning electron microscope was used to examine the foam BSP particles. The samples were coated with a very thin layer of gold, using sputter coater. The sample was then placed

inside the microscope's vacuum column through an air-tight door and examined at various magnifications.

5. RESULTS AND DISCUSSION

5.1. Effects of Carrier Matrix

Throughout the results section and remaining parts of the thesis the term volumetric loading rate refers to volumetric loading rate of sulphate and the term volumetric reduction rate refers to volumetric reduction rate of sulphate. Three continuous experimental runs were performed with three different carrier matrices namely, sand, foam biomass support particle (foam BSP) and glass beads. The employed carrier matrices had different specific surface areas and structure and as a result, employment of these carriers resulted in different total surface areas for establishment of biofilm and different void volumes in the bioreactor. Table 5.1 summarises the important parameters of the bioreactors packed with different matrices.

Table 5.1 Surface area and void volume for bioreactors operating with different carrier matrices

Carrier matrix	Specific surface area (m ² /g)	Total weight of carrier matrix (g)	Total surface area in the bioreactor (m ²)	Void volume (mL)
Glass Beads	0.001 ⁺	475	0.6	110
Foam BSP	0.216*	15	3.2	230
Sand	0.321*	452	145.1	60

*Specific surface area was measured twice. The average value of specific surface areas (Foam BSP: 0.306 and 0.126 m²/g; sand: 0.269 and 0.374 m²/g) are included in table. ⁺The specific surface area for glass bead was determined twice and the value which was close to theoretical value is included in the table.

The bioreactors void volume was used to calculate the residence time, volumetric loading rate, volumetric reduction rate and utilization rate of lactate, as well as production rate of acetate.

5.1.1. Performance of the Bioreactor Packed with Foam BSP

After inoculating the bioreactor with SRB culture and following a lag phase of three days, the residual sulphate concentration in the bioreactor started to decrease and production of sulphide was initiated. The bioreactor was switched to continuous mode after complete conversion of sulphate was achieved in all the three ports. Figure 5.1 shows the stepwise increase in the feed flow rate as a function of time. As can be seen in Figure 5.1, the bioreactor was run for a period of 147 days. The decrease in overall volumetric reduction rate was observed at a flow rate of 62.5 mL/h (corresponding to a residence time of 3.7 h).

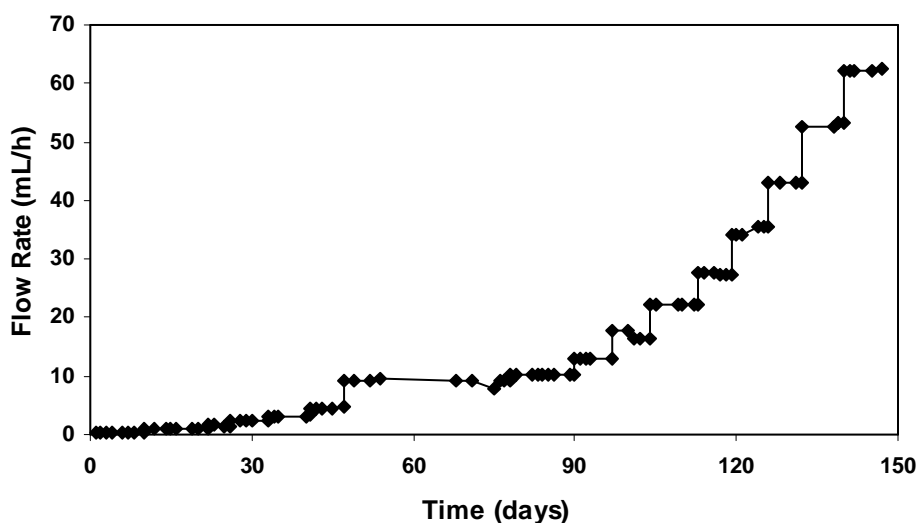
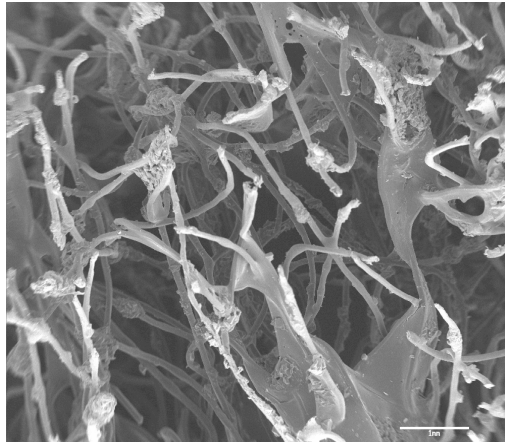
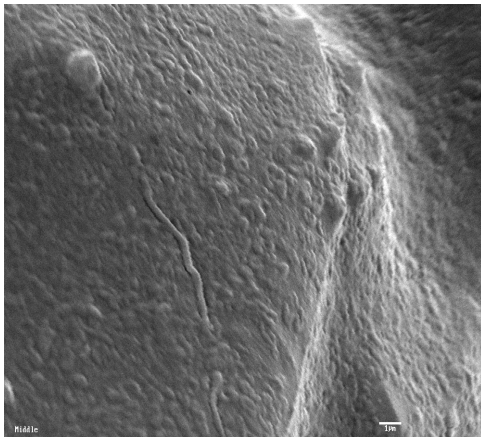


Figure 5.1 Variation of feed flow rate as a function of time for the bioreactor packed with foam BSP

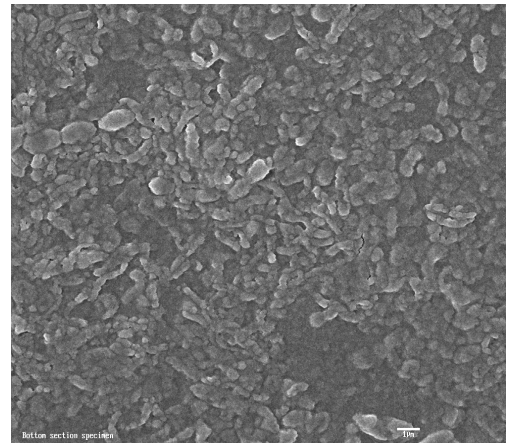
Examination of the foam BSP particles, removed from the bioreactor at the end of the experimental run, with scanning electron microscope indicated the successful passive immobilization of the cells and establishment of the biofilm. This was evident from the presence of extensive number of the cell on the surface of the particles (Figure 5.2). The fibrous and porous nature of the foam BSP matrix is shown in Figure 5.2a, which is the SEM of the fresh foam BSP carrier matrix. Figure 5.2b and 5.2c shows the bacterial cells on the surface of the foam BSP matrix taken from the middle and bottom parts of bioreactor, respectively.



(a)



(b)



(c)

Figure 5.2 Scanning electron micrographs of fresh foam BSP sample and the biofilm formed in the bioreactor packed with foam BSP

SEM for (a) -Fresh foam BSP (scale bar: 1mm), and samples taken from (b) – Middle and (c) - Bottom parts of the bioreactor (scale bar: 1µm).

5.1.1.1. Residual Sulphate and Produced Sulphide Concentrations and Conversion Profiles

Figure 5.3 shows the residual sulphate and produced sulphide concentration profiles in three different regions of bioreactor. The conversion profiles for different regions of bioreactor are shown in Figure 5.4. Conversions were calculated using the

initial and residual concentrations of sulphate or the initial concentration of sulphate and final concentration of the sulphide. In all experimental runs conversion calculated based on the concentration of produced sulphide was close to that calculated based on the sulphate concentrations, with the difference being equal or less than 15%. The sulphide produced inside the bioreactor may be present in different forms such as undissociated hydrogen sulphide in liquid and gas phase, HS^- and S^{2-} . So the measured hydrogen sulphide, which corresponds to various forms of sulphide in the liquid phase, may not account for the total sulphide produced. As a result the conversion calculated based on the initial and residual sulphate concentrations was used for assessment of bioreactor performance. Applying a low volumetric loading rate of 0.001 g/L-h, corresponding to a hydraulic residence time of 747.7 h, complete conversion of sulphate was observed in the lower part of the bioreactor (port 1). Increase of volumetric loading rate in the range 0.001 to 0.018 g/L-h (decrease of residence time from 747.7 to 68.4 h) led to increase of residual sulphate concentration from 0 to 0.48 g/L, and a decrease of conversion to around 60% in the lower part of the bioreactor. Further stepwise increase in volumetric loading rate led to lower conversions but decrease in conversion was not as sharp as that observed in the initial parts of the experiment. This could be attributed, possibly, to adaptation of bacteria to environmental conditions. In the middle part of the bioreactor (port 2) high conversions of 90-100% were observed at volumetric loading rates as high as 0.063 g/L-h (residence time 17.8 h), while in the upper part of the bioreactor (port 3) these high conversions were attained at volumetric loading rates up to 0.223 g/L-h (residence time 5.4 h). The highest applied volumetric loading rate was 0.32 g/L-h (residence time 3.7 h), resulting in 29.2, 42.3 and 48.6% conversions in the lower, middle and upper parts of the bioreactor, respectively.

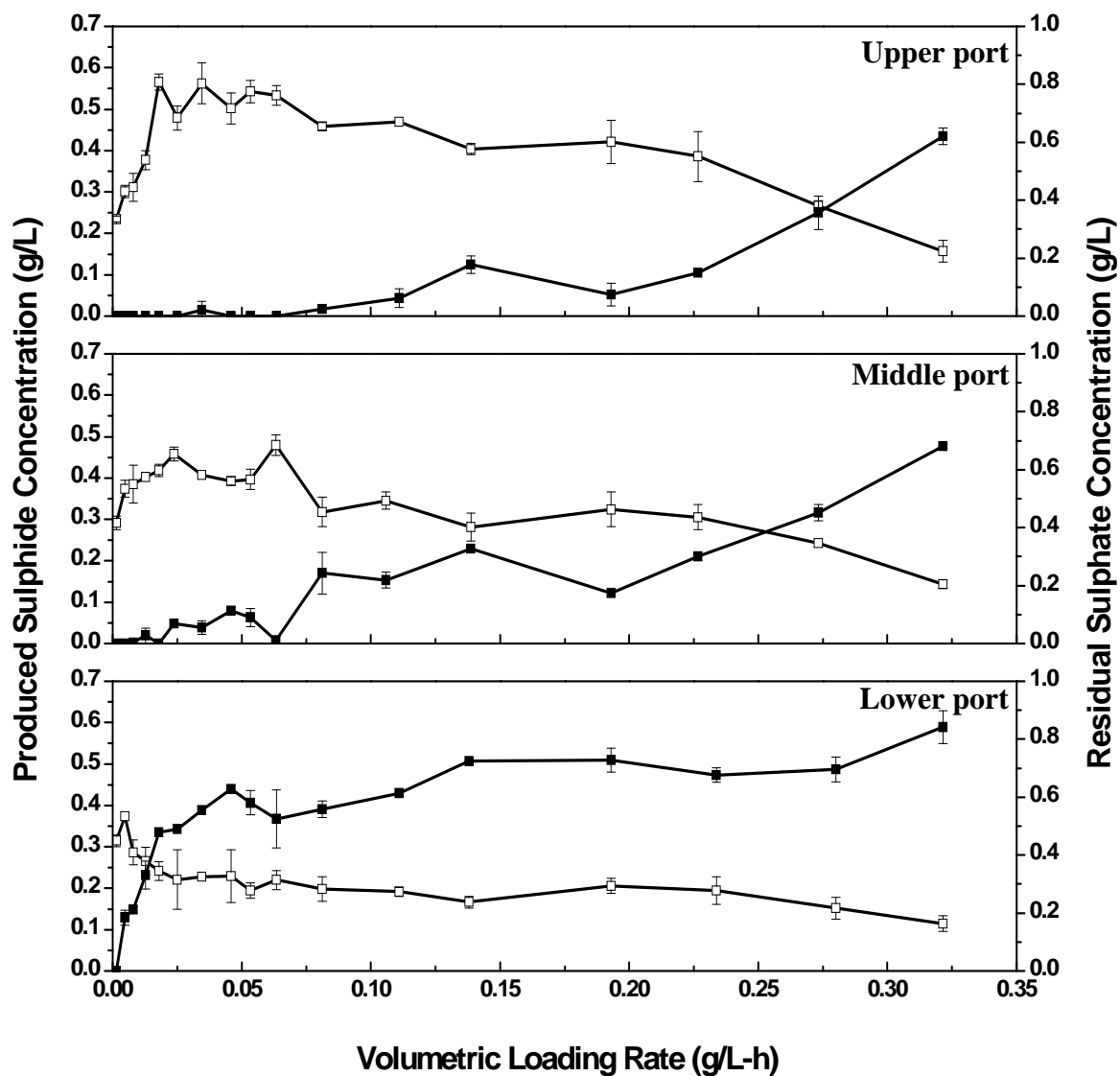


Figure 5.3 Residual sulphate and produced sulphide concentration profiles for the bioreactor packed with foam BSP
 (□)-Produced sulphide concentration profile, (■) – Residual sulphate concentration profile. An error bar was plotted for all the steady state points. In some cases the error bar is not visible as the associated error was small.

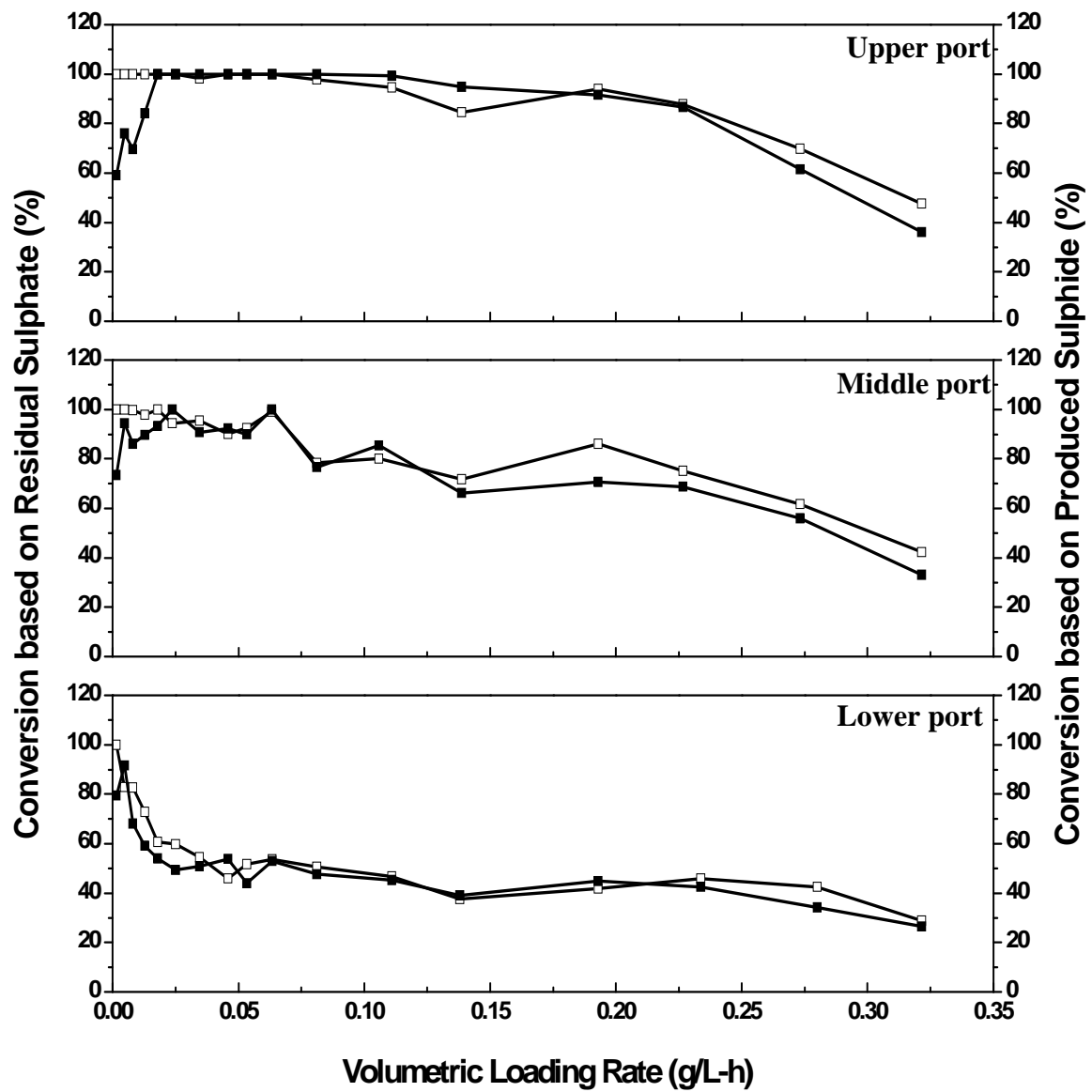


Figure 5.4 Conversion profiles calculated based on sulphate and sulphide concentrations for the bioreactor packed with foam BSP
 (□)-Conversion based on sulphate, (■) – Conversion based on sulphide.

5.1.1.2. Organic Acids (Lactate and Acetate) Concentration Profiles

The profiles of lactate and acetate concentrations in the different regions of bioreactor are shown in Figure 5.5. As can be seen, at a constant volumetric loading rate the residual concentration of lactate decreased along the length of the bioreactor, while the concentration of produced acetate increased, indicating the progress of the reaction as reactants passed through the bioreactor. The dependency of residual concentration of lactate on volumetric loading rate had a pattern similar to that for the residual sulphate concentration. Applying a volumetric loading rate of 0.001 g/L-h (the lowest value applied) the residual concentration of lactate was zero in all three ports. Considering the fact that the amount of provided lactate was around 45% higher than the value required for reduction of present sulphate, part of the provided lactate must have been used for the growth and activity of the heterotrophic population. The increase of volumetric loading rate in the range 0.001 to 0.081 g/L-h increased the residual concentration of the lactate to 2.5, 1.4 and 0.8 g/L in the lower, middle and upper parts of the bioreactor, respectively. Further increase of volumetric loading rate did not have a significant effect on the residual lactate concentration in the lower part of the bioreactor, while in the middle and upper parts the residual lactate concentration gradually increased to a final value around 2.3 g/L. The concentration of produced acetate increased along the length of the bioreactor with the highest concentrations of 1.6, 2.4 and 2.6 g/L observed at the lowest applied volumetric loading rate (0.001 g/L-h) for the lower, middle and upper ports, respectively.

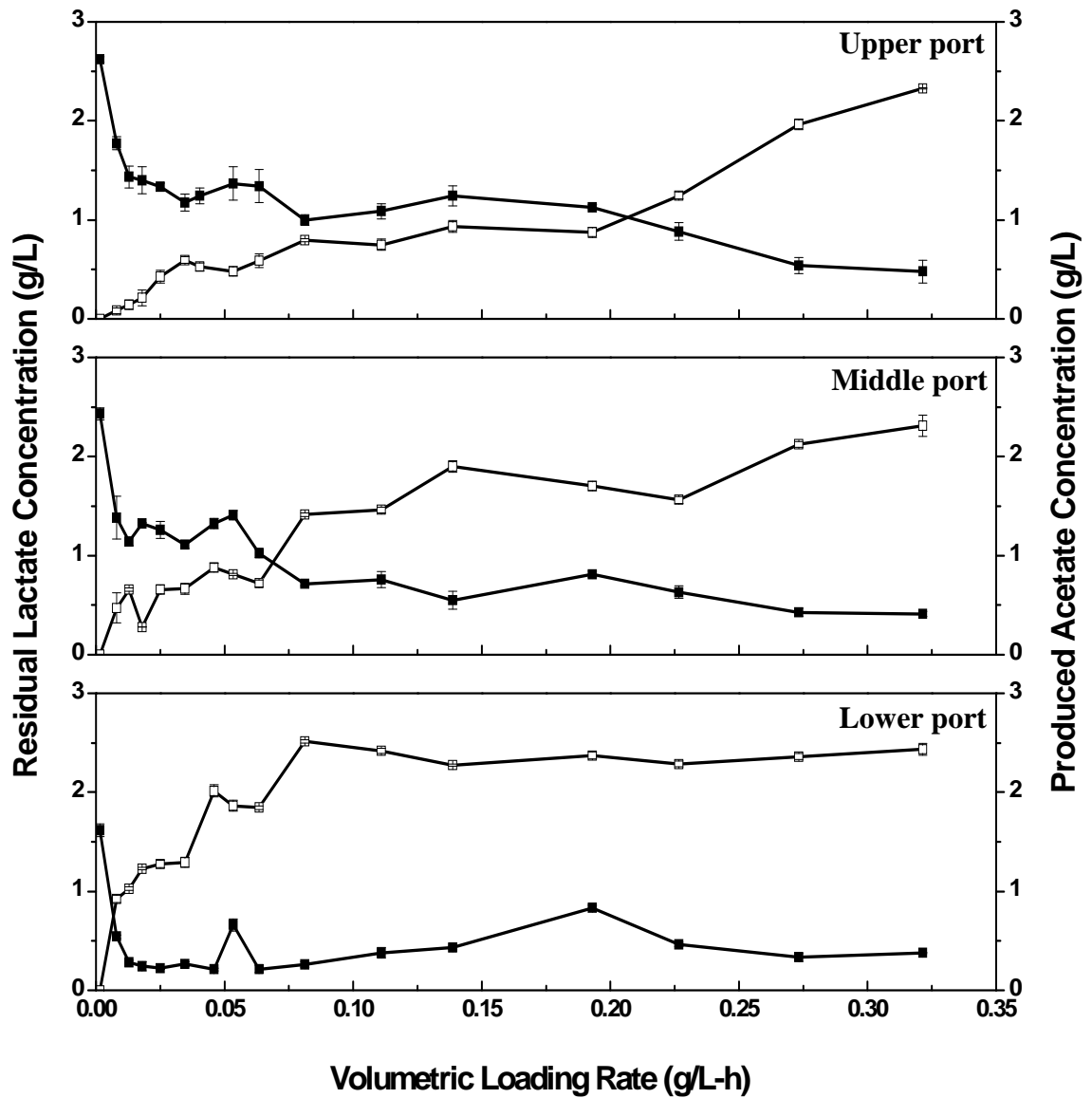


Figure 5.5 Residual lactate and produced acetate concentration profiles for the bioreactor packed with foam BSP
 (□)- Residual lactate concentration profile, (■) – Produced acetate concentration profile.

An increase of the volumetric loading rate from 0.001 to 0.013 g/L-h decreased the concentration of acetate in all three ports sharply. Further increase in

volumetric loading rate resulted in gradual decrease of acetate concentration in the middle and upper parts of the bioreactor, while the effect on the lower part was not significant.

5.1.1.3. Volumetric Reduction Rate, Lactate Utilization and Acetate Production Rates

The dependency of sulphate conversion and volumetric reduction rate, lactate utilization rate and acetate production rate on the volumetric loading rate are shown in Figures 5.6 and 5.7, respectively. The volumetric reduction rate, utilization rate of lactate and production rate of acetate were calculated, using the initial and final concentrations of each compound and the residence time calculated on the basis of feed flow rate and bioreactor void volume. As can be seen in Figure 5.6, for the volumetric loading rates in the range of 0.001 to 0.227 g/L-h (residence time ranging from 747.7 to 5.4 h) the overall conversion (determined based on the data collected from the upper port) was in the range 90-100%, with the lower values observed at higher volumetric loading rates. The increase of volumetric loading rates over this range led to a linear increase in volumetric reduction rate with the highest volumetric reduction rate of 0.202 g/L-h observed at a volumetric loading rate of 0.227 g/L-h. The corresponding residence time was 5.4 h and the conversion of sulphate was around 89%. Further increase of volumetric loading rate (decrease of residence time) led to a sharp decrease in sulphate conversion and volumetric reduction rate. The highest volumetric loading rate applied in this experimental run was 0.325 g/L-h (residence time of 3.7 h), resulting in a conversion of 48.5% and a reduction rate of 0.156 g/L-h.

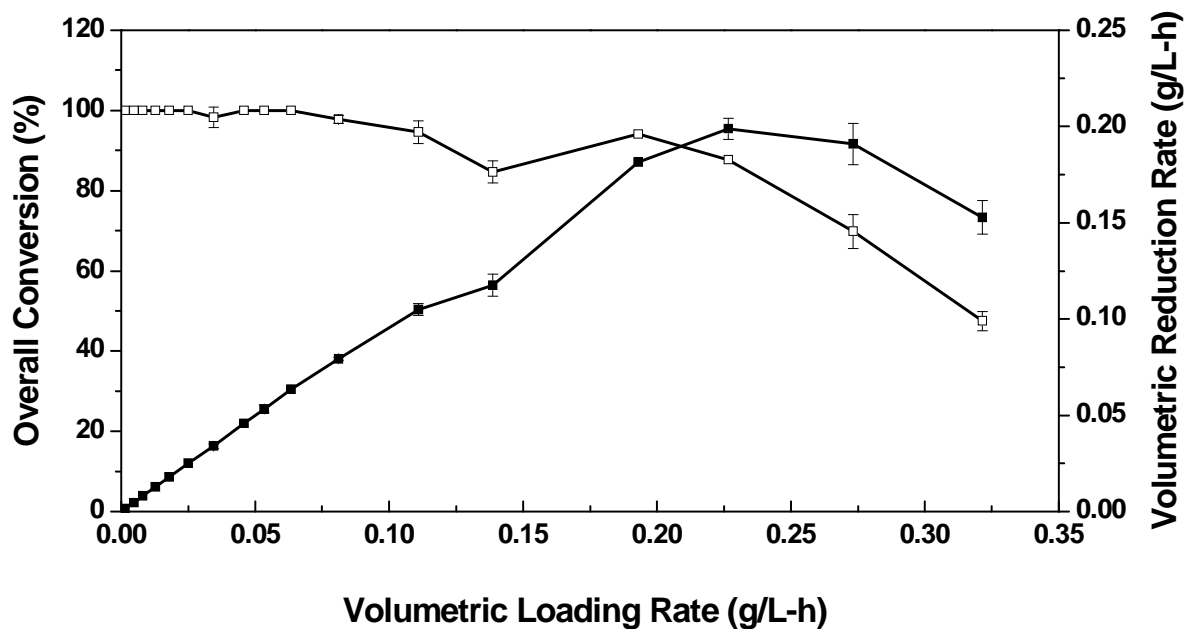


Figure 5.6 Overall conversion and volumetric reduction rate profiles for the bioreactor packed with foam BSP
 (□)-Overall conversion profile and (■) – Overall volumetric reduction rate profile.

Similar to the pattern observed for volumetric reduction rate, the utilisation rate of lactate and acetate production rate were dependent on volumetric loading rate (Figure 5.7), with the maximum lactate utilization rate of 0.272 g/L-h and maximum acetate production rate of 0.174 g/L-h were both achieved at a volumetric loading rate of 0.193 g/L. Further increase in volumetric loading rate led to a decrease in lactate utilisation and acetate production rates.

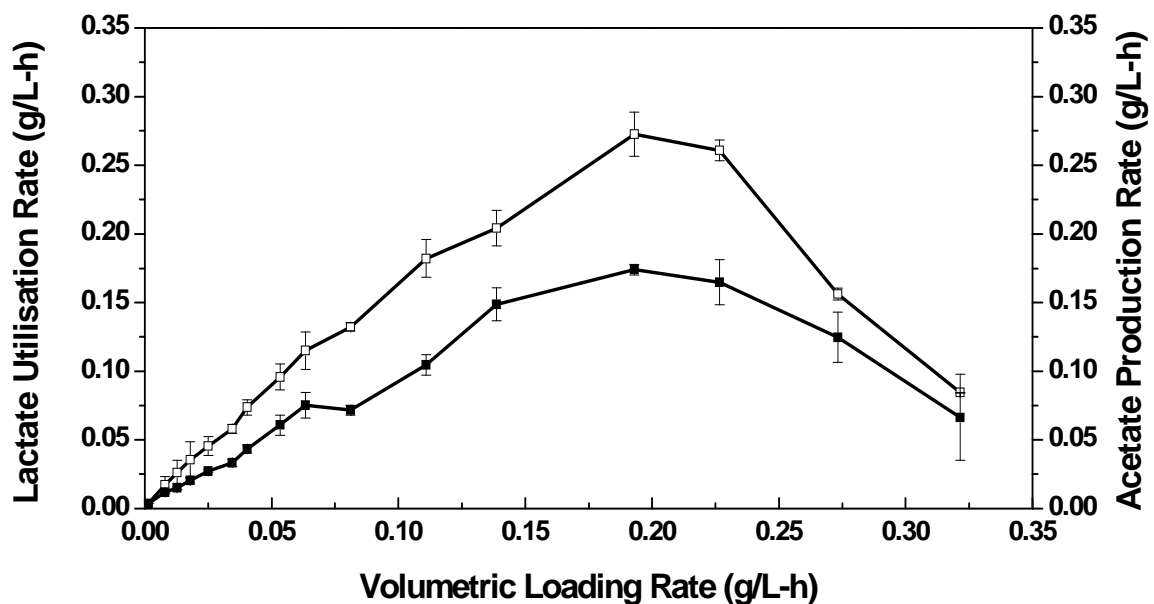


Figure 5.7 Profiles for overall lactate utilisation and acetate production rates for the bioreactor packed with foam BSP

(□) - Overall lactate utilisation rate profile and (■) – Overall acetate production rate profile.

5.1.2. Performance of Bioreactor Packed With Glass Bead

A lag phase of two days was observed after inoculation of bioreactor. This was followed by the reduction of sulphate to sulphide. Once complete conversion of sulphate to sulphide was achieved the bioreactor was switched to continuous operation. Figure 5.8 shows the stepwise increase in the feed flow rate as a function of time. The bioreactor was run for a period of 71 days. The decrease in overall volumetric reduction rate was observed at a comparatively low flow rate of 6.0 mL/h (corresponding to a residence time of 18.3 h).

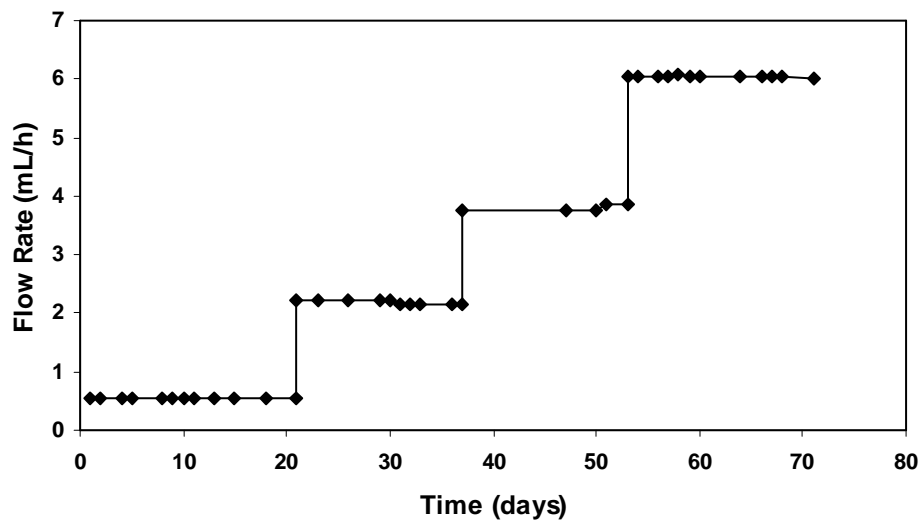


Figure 5.8 Variation of feed flow rate as a function of time for the bioreactor packed with glass bead

5.1.2.1. Residual Sulphate and Produced Sulphide Concentrations and Conversion Profiles

Figure 5.9 shows the sulphide and sulphate concentration profiles for the three regions of the bioreactor operating with glass bead. Figure 5.10 shows the conversion profile calculated based on residual sulphate concentration. The produced sulphide and residual sulphate concentration profiles had a pattern similar to that observed in the bioreactor operated with foam BSP as a carrier matrix. As the volumetric loading rate was increased the residual sulphate concentration began to increase with accompanying decrease in sulphide concentration. Applying volumetric loading rates up to 0.01 g/L-h (corresponding residence time: 102.3 h) the residual sulphate concentration remained zero in the lower part of the bioreactor. When the volumetric loading rate was increased to 0.022 g/L-h, a sharp increase in the residual sulphate concentration was observed and

the conversion dropped from 100% to 46.4%. Further increase in volumetric loading rates led to gradual decrease in conversion and increase in residual sulphate concentration. In the middle part of bioreactor the residual sulphate concentration remained zero for volumetric loading rates up to 0.022 g/L-h (residence time of 51.4 h). When the volumetric loading rate was increased above 0.022 g/L-h a sharp increase in the residual sulphate concentration was detected and the conversion decreased sharply from to 45.9%. Complete conversion was observed in the upper part of the bioreactor up to a volumetric loading rate of 0.043 g/L-h (residence time of 28.6 h). At the maximum applied volumetric loading rate of 0.0682 g/L-h (residence time of 18.3 h) the residual sulphate concentration was around 1.1 g/L (conversion: 11.6%), 0.95 g/L (conversion: 23.8%) and 0.65 g/L (conversion: 48.38%) in the lower, middle and upper parts of the bioreactor, respectively. It should be pointed out that the adjustment of feed sulphate concentration at an exact value of 1.0 g/L was impractical and in some cases feed sulphate concentration varied between 1.0 to 1.25 g/L. The maximum produced sulphide concentrations detected were 0.52, 0.57 and 0.59 g/L at the lower, middle and upper parts of the bioreactor, respectively. This maximum concentration of sulphide was detected at the volumetric loading rate of 0.009 g/L-h for the lower part and at 0.022 g/L-h for the middle and upper parts of the bioreactor.

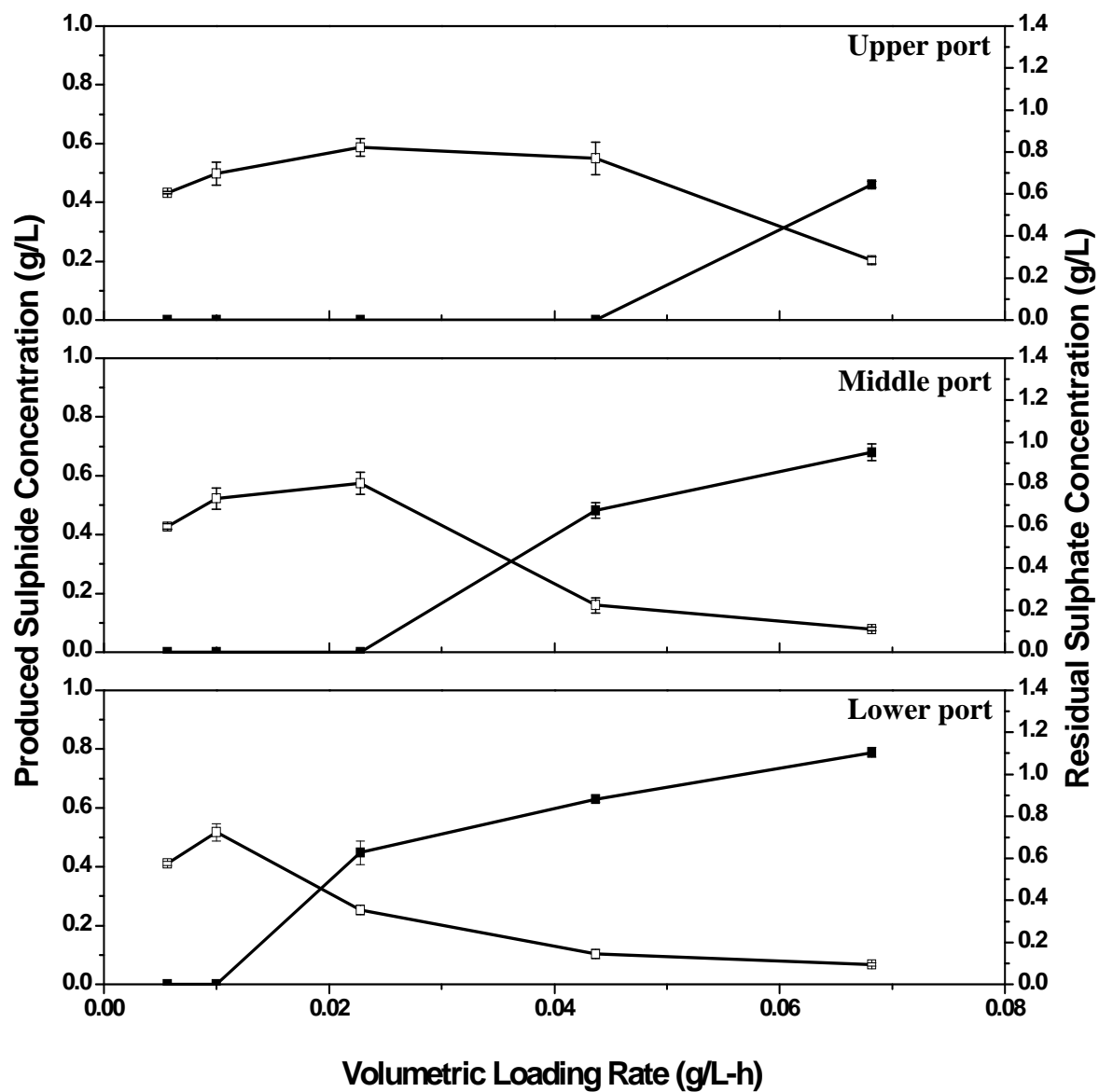


Figure 5.9 Residual sulphate and produced sulphide concentration profiles for the bioreactor packed with glass bead
 (□)-Produced sulphide concentration profile and (■) – Residual sulphate concentration profile.

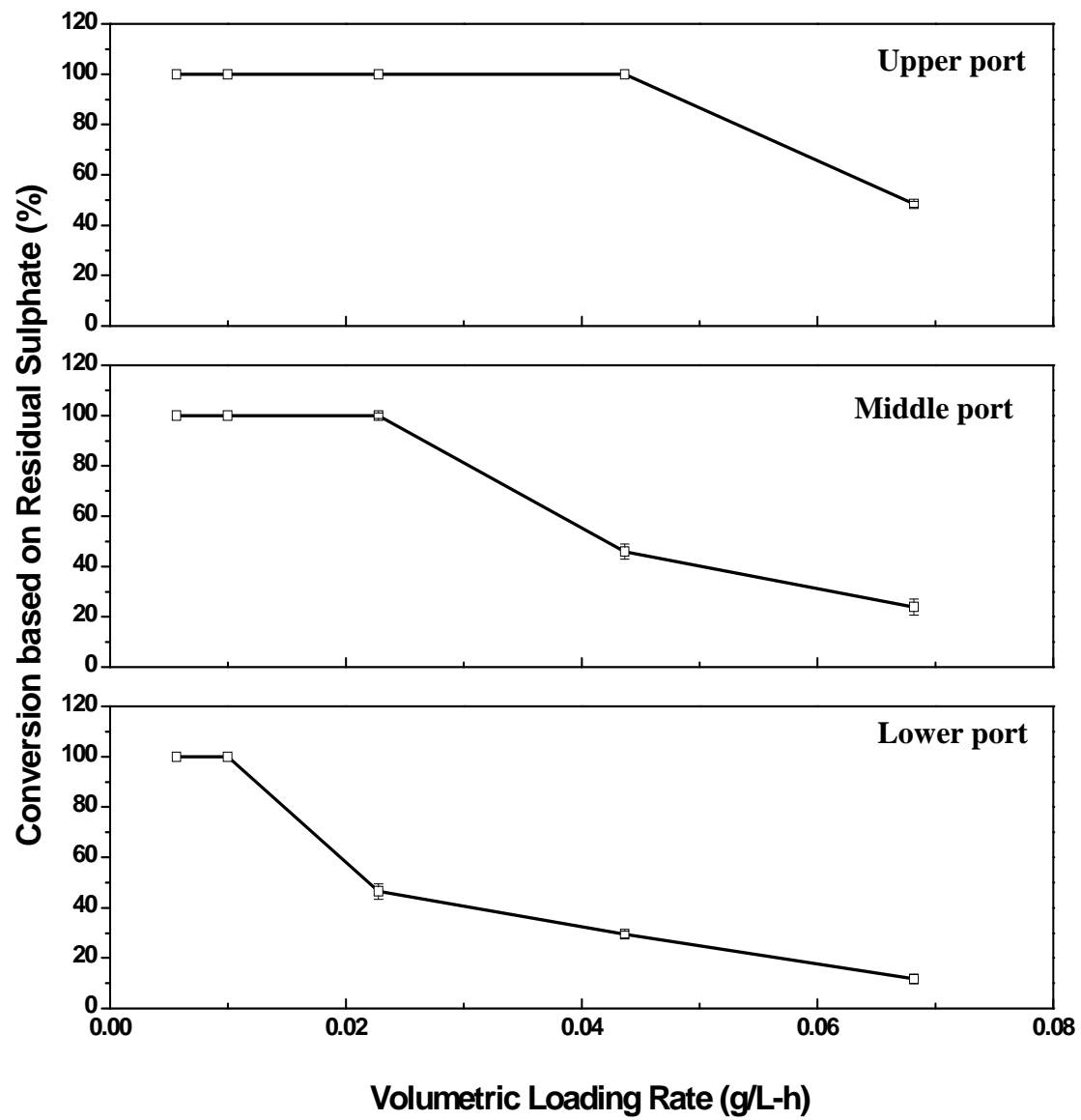


Figure 5.10 Conversion profile calculated based on sulphate concentration for the bioreactor packed with glass bead

5.1.2.2. Organic Acids (*Lactate and Acetate*) Profiles

Figure 5.11 shows the residual lactate and produced acetate concentration profiles for three regions of the bioreactor. The lactate and acetate concentration profiles (Figure 5.11) resemble the residual sulphate and produced sulphide concentration profiles (Figure 5.9). Operating the bioreactor at a volumetric loading rate of 0.006 g/L-h the residual lactate concentration was zero in the second and third ports. The residual lactate concentration increased gradually due to employment of higher volumetric loading rates. As can be seen in Figure 5.9 the residual sulphate concentration profile followed a similar pattern, indicating the coupling of sulphate reduction to that of lactate utilization. At the highest applied volumetric loading rate (0.068 g/L-h) the residual lactate concentration was found to be 2.55, 2.5 and 1.88 g/L in the lower, middle and upper parts of bioreactor, respectively.

As the residual lactate concentration increased with the increase in volumetric loading rates, the produced acetate concentration decreased. The highest produced acetate concentrations observed were 2.0, 1.79 and 1.81 g/L at the lower middle and upper parts of the bioreactor, respectively, at a volumetric loading rate of 0.009 g/L-h. At the highest applied volumetric loading rate of 0.068 g/L-h, the produced acetate concentration was found to be 0.09, 0.18 and 0.54 g/L in the lower, middle and upper parts of the bioreactor, respectively.

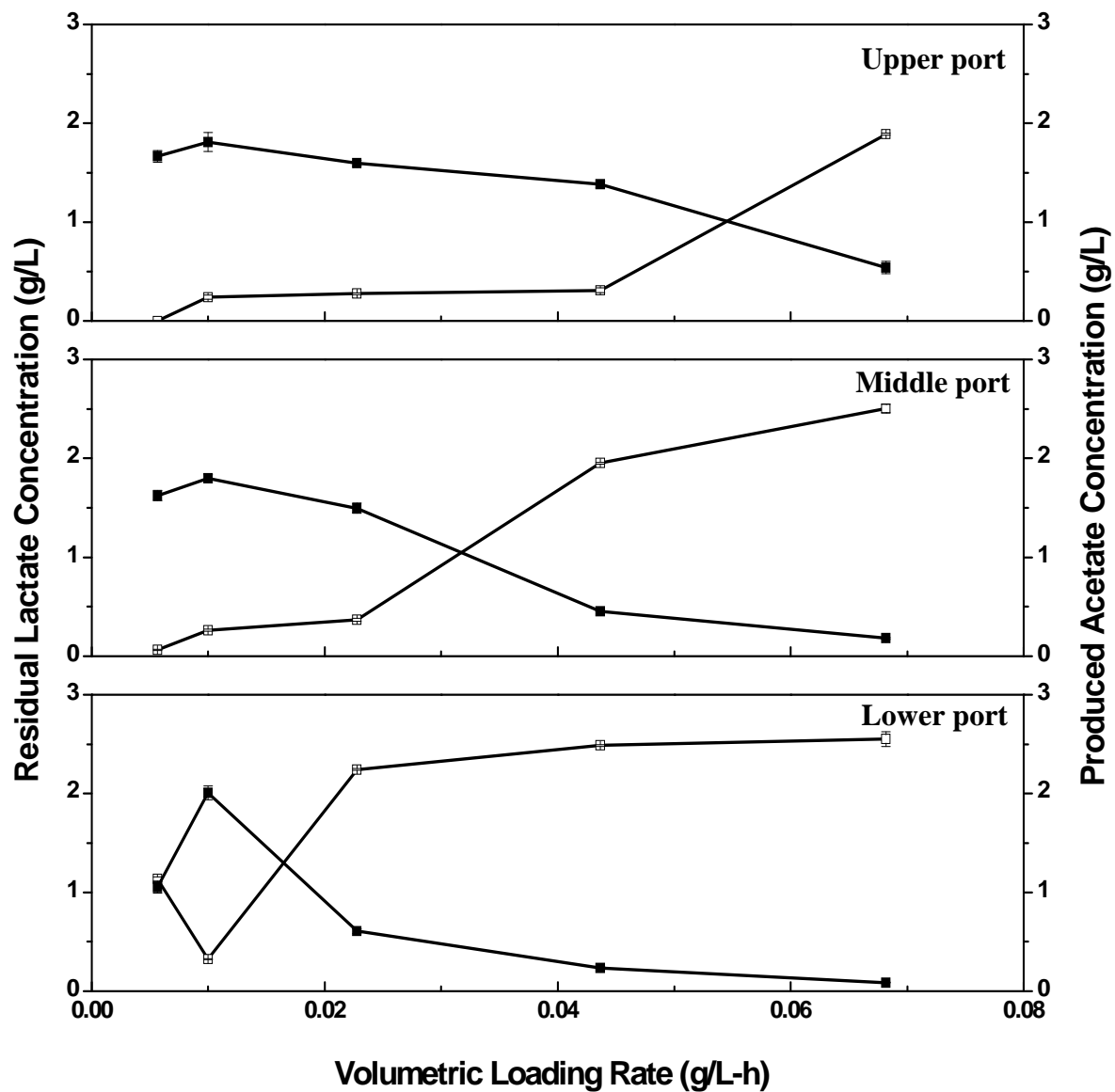


Figure 5.11 Residual lactate and produced acetate concentration profiles for the bioreactor packed with glass bead
 (□)- Residual lactate concentration profile and (■) – Produced acetate concentration profile.

5.1.2.3. Volumetric Reduction Rate, Lactate Utilization and Acetate Production Rates

Figure 5.12 represents the overall conversion and volumetric reduction rate for the bioreactor operated with glass bead as carrier matrix. Overall conversion was 100% for volumetric loading rate up to 0.044 g/L-h (residence time: 28.6 h). The overall volumetric reduction rate was found to increase linearly with the increase in volumetric loading rate up to 0.044 g/L-h. When the volumetric loading rate was increased to 0.068 g/L-h (residence time: 18.3 h), a decrease in overall volumetric reduction rate was observed with its value dropping to 0.032 g/L-h. The highest volumetric reduction rate was 0.044 g/L-h, observed at a volumetric loading rate of 0.044 g/L-h, with a corresponding conversion of 100%.

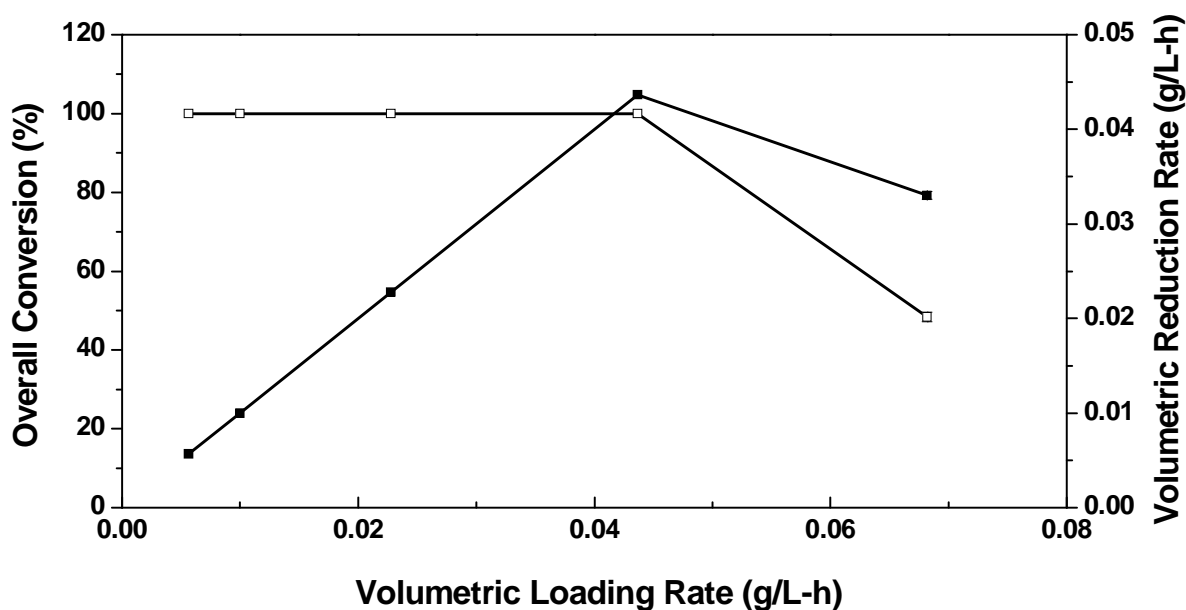


Figure 5.12 Overall conversion and volumetric reduction rate profiles for the bioreactor packed with glass bead

(□)-Overall conversion profile and (■) – Overall volumetric reduction rate profile.

The overall lactate utilisation and acetate production rates profiles are shown in Figure 5.13. As volumetric loading rate was increased, the overall lactate utilisation and the acetate production rates increased linearly, similar to sulphate volumetric reduction rate profile. The highest value of lactate utilisation rate (0.081 g/L-h) and acetate production rate (0.048 g/L-h) were observed at the same volumetric loading rate at which the highest sulphate volumetric reduction rate was achieved. When the volumetric loading rate was increased to 0.068 g/L-h, a drop in both lactate utilisation and acetate production rates were observed.

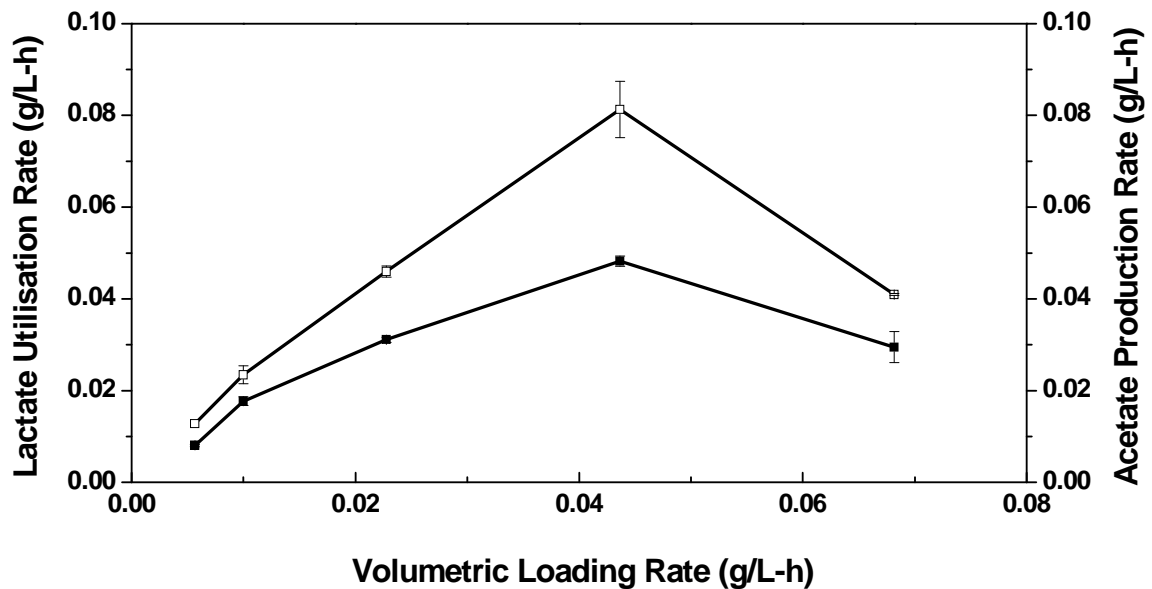


Figure 5.13 Profiles for overall lactate utilisation and acetate production rates for the bioreactor packed with glass bead
 (□) - Overall lactate utilisation rate profile and (■) – Overall acetate production rate profile.

5.1.3. Performance of Bioreactor Packed with Sand

The performance of the bioreactor was also investigated using sand (-50+70 mesh size; average diameter of 225 μm) as a carrier matrix with the feed sulphate concentration of 1.0 g/L. The initial flow rate employed was 0.30 mL/h (corresponding to a residence time of 200.0 h) which was increased stepwise to a maximum flow rate of 134.7 mL/h (corresponding to a residence time of 0.4 h) as shown in Figure 5.14, before a reduction in volumetric reduction rate was seen. The final flow rate (134.7 mL/h) in this case was much higher than the maximum flow rates employed for the bioreactors operated with foam BSP and glass bead. The total time required to complete this entire continuous run was 119 days.

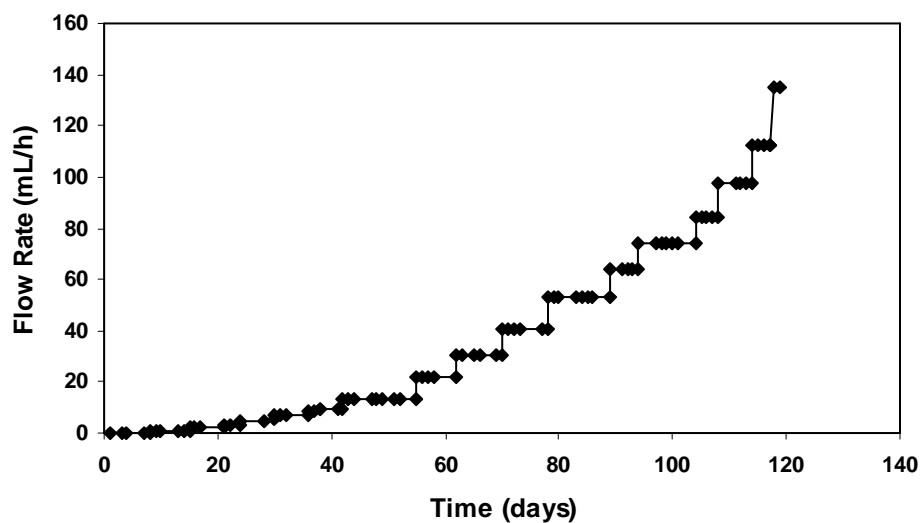


Figure 5.14 Variation of feed flow rate as a function of time for the bioreactor fed with a medium containing 1.0 g/L of sulphate and packed with sand

5.1.3.1. Protein Concentration Profiles

As indicated earlier, in the experiments with sand as a carrier matrix protein concentration in the liquid phase was measured and used as an indication of the freely suspended biomass level in the bioreactor. Figure 5.15 summarises the protein concentration profiles for three different regions of the bioreactor. The protein concentration was found to pass through a maximum as the volumetric loading rate was increased. However, the maximum protein concentration in each port was observed at different volumetric loading rates. The protein concentration in the lower port of the bioreactor reached the maximum value of 70.1 mg/L at a volumetric loading rate of 0.154 g/L-h (residence time: 6.6 h), while the maximum protein concentrations in the middle and upper parts of the bioreactor, 122.3 and 140.3 mg/L, were obtained at volumetric loading rates of 0.424 g/L-h (residence time: 2.8 h) and 1.43 g/L-h (residence time: 0.8 h), respectively. The increase in the volumetric loading rate above the given values led to the decrease in the protein concentration in the corresponding parts of the bioreactor.

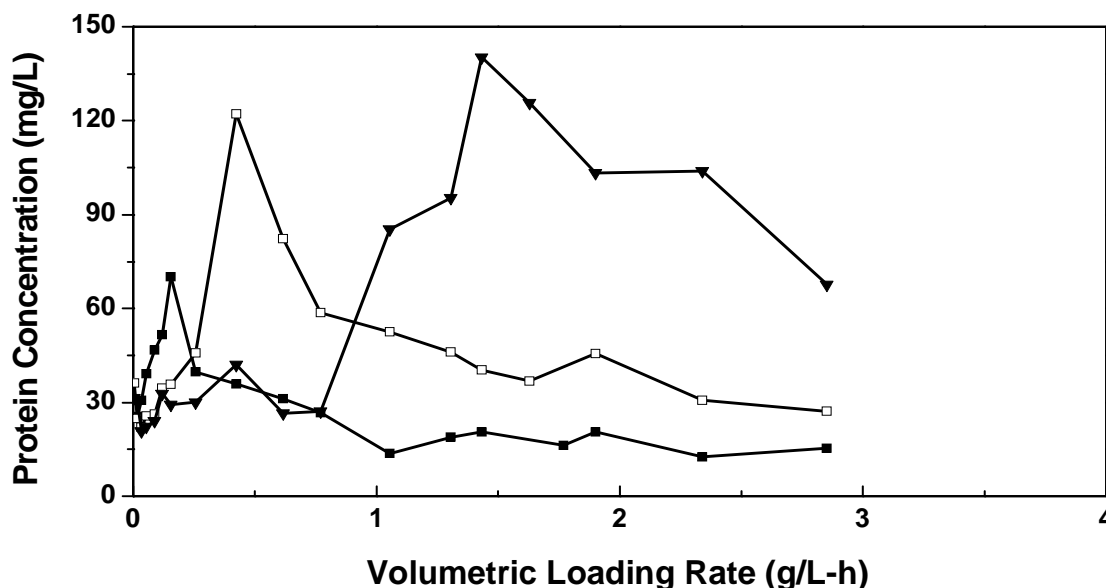


Figure 5.15 Liquid phase protein concentration profiles for the bioreactor fed with a medium containing 1.0 g/L of sulphate and packed with sand
 (■)-Lower, (□)-Middle and (▼)-Upper parts.

5.1.3.2. Residual Sulphate and Produced Sulphide Concentrations and Conversion

Profiles

Figure 5.16 and Figure 5.17 summarize the profiles for the residual sulphate and the produced sulphide concentrations and the conversion profile as a function of volumetric loading rates for three different regions of the bioreactor, respectively. Complete conversion of sulphate to sulphide was observed in the lower part of the bioreactor up to the volumetric loading rate of 0.119 g/L-h (residence time: 8.6 h). Increasing of volumetric loading rates in the range 0.119 to 0.615 g/L-h (residence time: 1.96 h), decreased the conversion from 100% to 46% in the lower part of the bioreactor. In the middle and upper parts of the bioreactor the residual sulphate concentration

remained zero for volumetric loading rates up to 0.425 g/L-h (residence time: 2.7 h) and 0.771 g/L-h (residence time: 1.5 h), respectively. When the volumetric loading rate was increased in the range 0.615 to 1.626 g/L-h, the conversion in the middle part was decreased from 96 to 21%. Further increase in the volumetric loading rate led to a gradual decrease in the conversion. In the upper part of the bioreactor, when the volumetric loading rate was increased above 0.771 g/L-h, a gradual decrease in the conversion was observed. With the highest applied volumetric loading rate of 2.85 g/L-h (residence time: 0.5 h), the residual sulphate concentration was 1.12 g/L (11.8% conversion), 1.07 g/L (15.6% conversion) and 0.53 g/L (58.2% conversion) in the lower, middle and upper parts of bioreactor, respectively. The maximum produced sulphide concentration observed was 0.536 g/L, 0.552 g/L and 0.568 g/L in the lower, middle and upper parts of the bioreactor, respectively. These maximum sulphide concentrations were observed at the volumetric loading rate of 0.119, 0.033 and 0.153 g/L-h for the lower, middle and upper parts of the bioreactor, respectively.

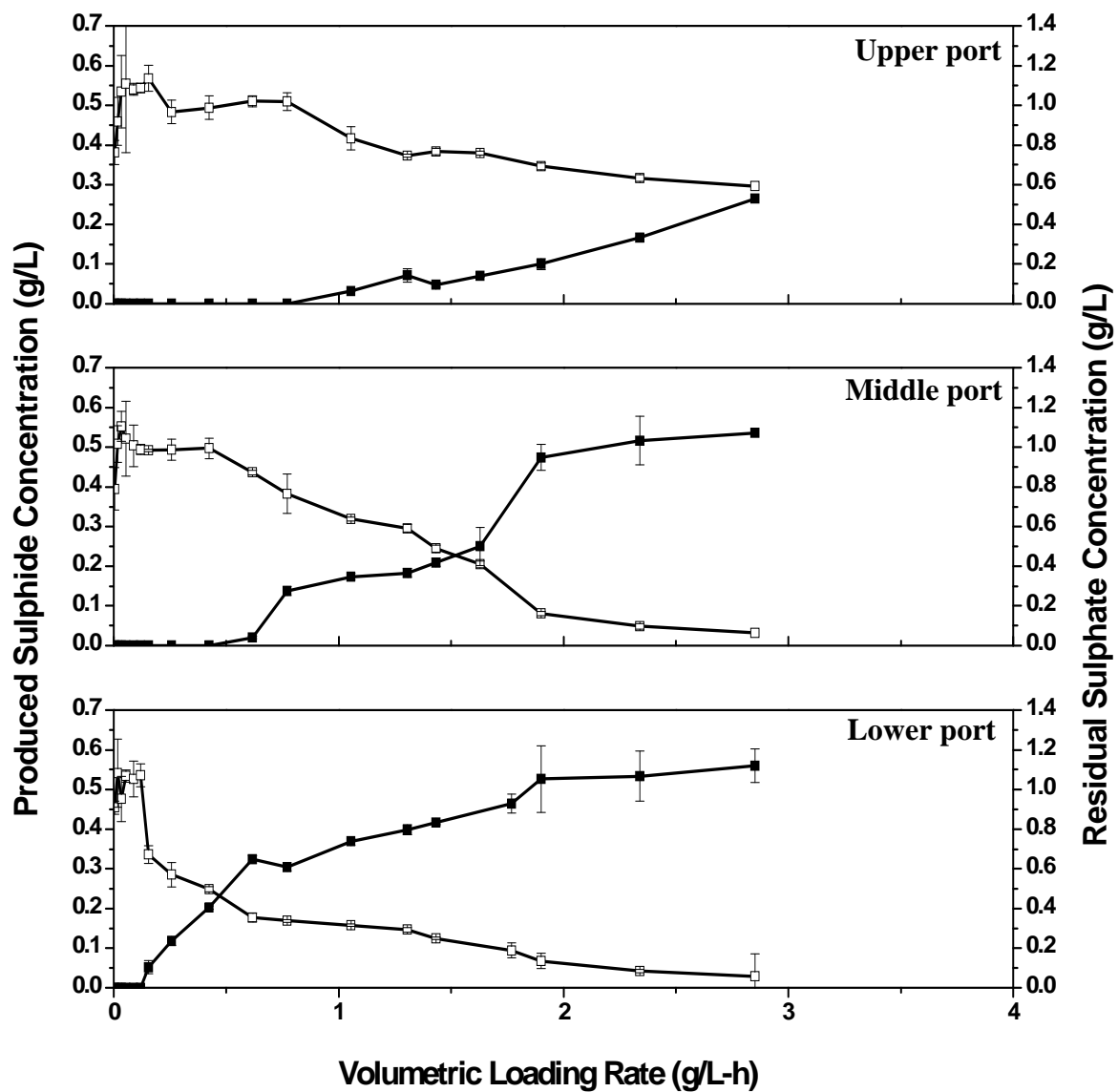


Figure 5.16 Residual sulphate and produced sulphide concentration profiles for the bioreactor fed with a medium containing 1.0 g/L of sulphate and packed with sand (□)-Produced sulphide concentration profile and (■) – Residual sulphate concentration profile.

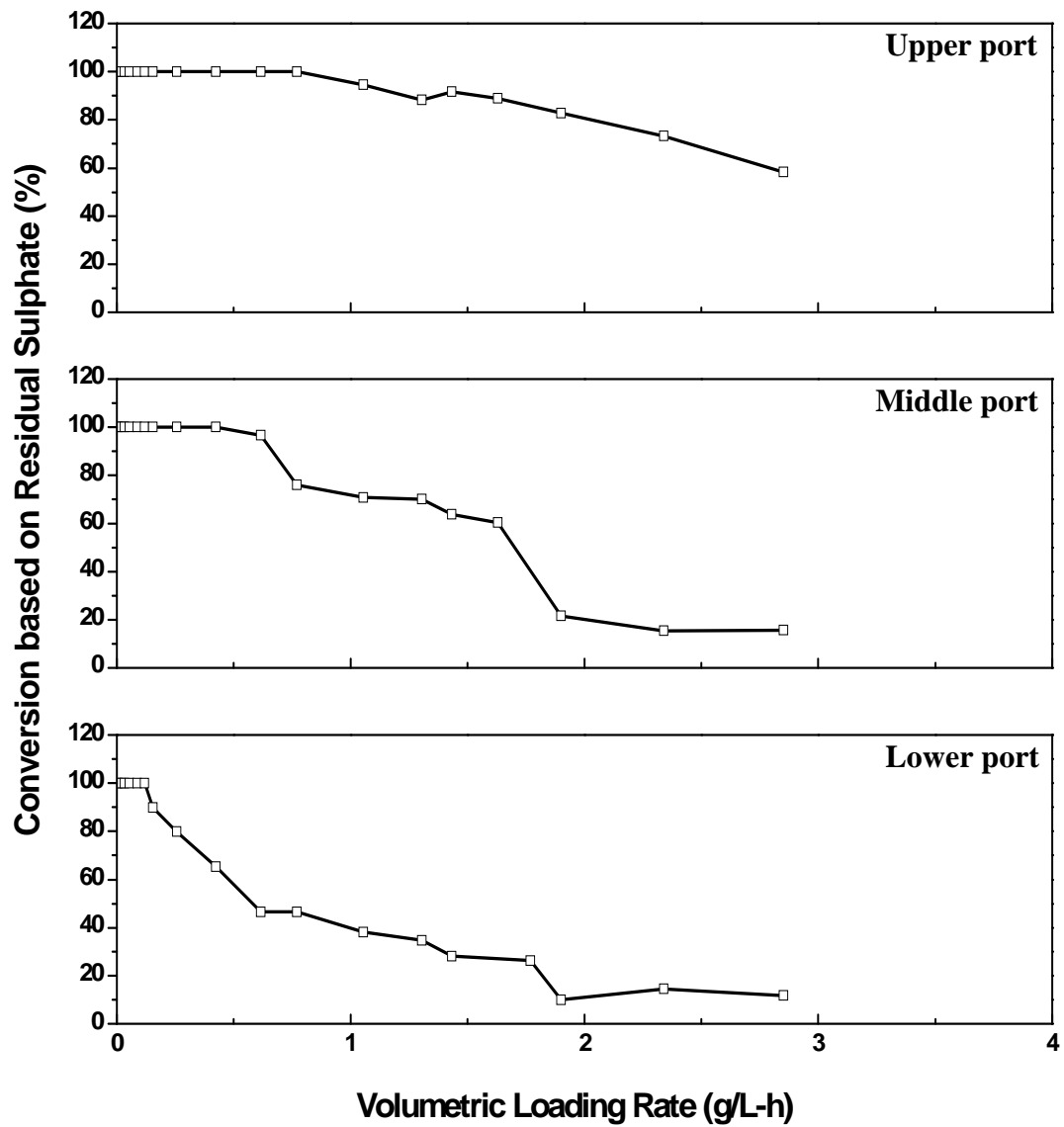


Figure 5.17 Conversion profile calculated based on sulphate concentration for the bioreactor fed with a medium containing 1.0 g/L of sulphate and packed with sand

5.1.3.3. Organic Acids (Lactate and Acetate) Concentration Profiles

The lactate and acetate concentration profiles are shown in Figure 5.18. The coupling of sulphate reduction, lactate utilization and acetate production was also observed in this bioreactor. From Figure 5.18 it can be seen that the provided lactate was completely consumed at the volumetric loading rate of 0.033 g/L-h (residence time: 30.7 h) in the upper part of the bioreactor. Apart from this, the residual lactate concentration remained always above zero in all three regions of the bioreactor. At a constant applied volumetric loading rate the lactate concentration, similar to the residual sulphate concentration, was found to decrease along the length of the bioreactor. In the lower and middle parts of the bioreactor for volumetric loading rates up to 0.154 g/L-h lactate concentration was below 0.6 g/L. The increase of volumetric loading rate above 0.154 g/L-h led to a linear increase in lactate concentration, with the rate of increase in the lower part being faster. Similar to what observed for the residual sulphate concentration profile, no sharp increase in the residual lactate concentration occurred in the upper part of the bioreactor until a volumetric loading rate of 2.85 g/L-h was applied. At this volumetric loading rate (highest applied) the residual lactate concentration was found to be 2.45, 2.26 and 1.83 g/L in the lower, middle and upper parts of the bioreactor, respectively. The highest acetate concentration for the lower, middle and upper parts of the bioreactor was 1.36, 1.47 and 1.58 g/L, observed at volumetric loading rates of 0.087, 0.257 and 0.615 g/L-h, respectively.

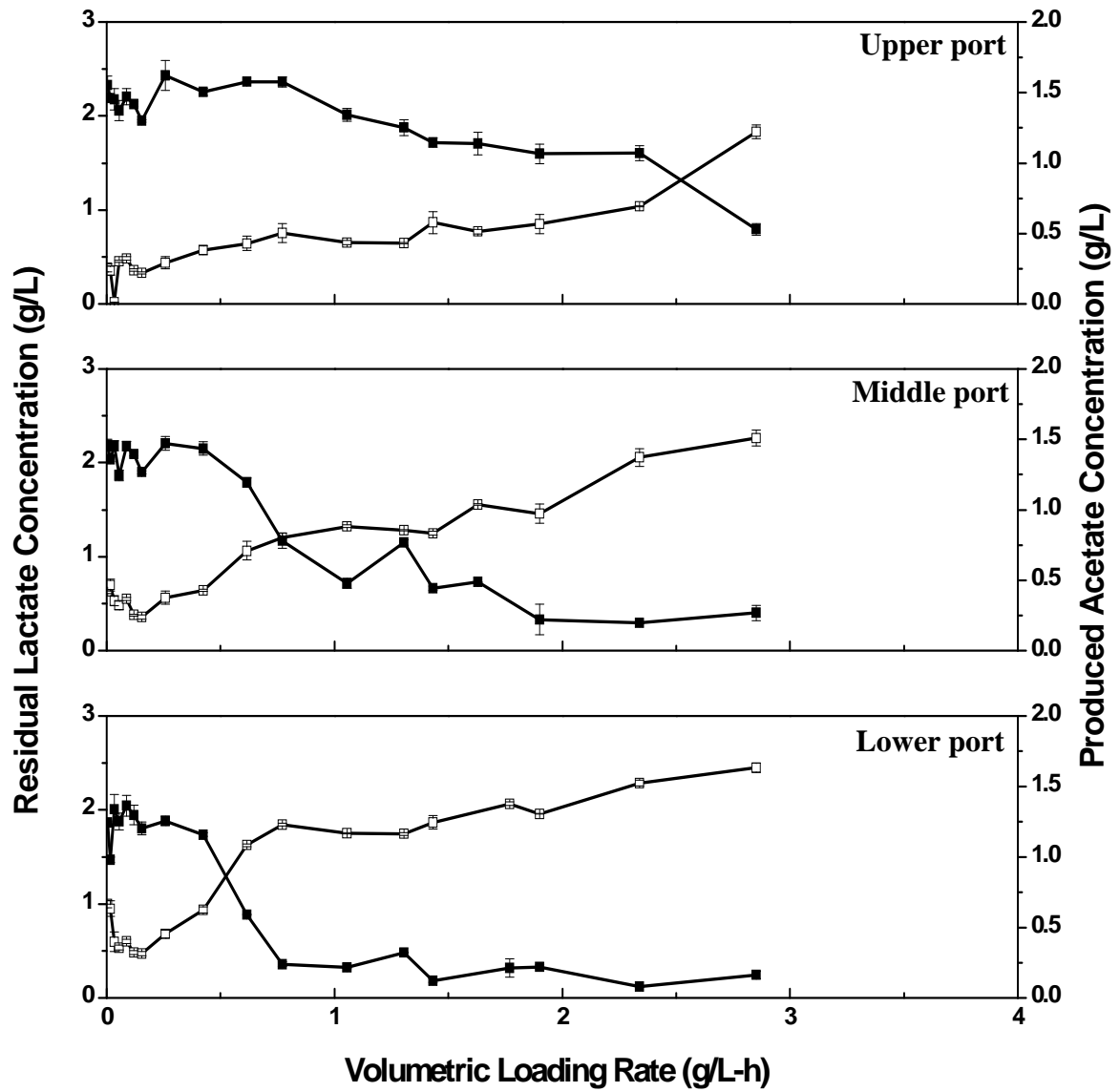


Figure 5.18 Residual lactate and produced acetate concentration profiles for the bioreactor fed with a medium containing 1.0 g/L of sulphate and packed with sand (□)- Residual lactate concentration profile, (■) – Produced acetate concentration profile.

5.1.3.4. Volumetric Reduction Rate, Lactate Utilization and Acetate Production Rates

The overall conversion and volumetric reduction rate was determined for the bioreactor and the profiles are shown in Figure 5.19. The overall volumetric reduction rate was found to increase linearly with the increase in the volumetric loading rate. The overall conversion was high (100 - 89%) up to a volumetric loading rate of 1.629 g/L-h (residence time: 0.8 h). The maximum overall volumetric reduction rate observed was 1.71 g/L-h, at a volumetric loading rate of 2.34 g/L-h (residence time: 0.5 h), with a corresponding conversion of 73.2%. When the residence time was decreased below 0.5 h, a decline in volumetric reduction rate was observed. At the highest applied volumetric loading rate of 2.85 g/L-h (residence time: 0.4 h), the overall volumetric reduction rate and conversion were dropped to 1.65 g/L-h and 58.2%, respectively. The lowest residence time for which complete conversion of sulphate to sulphide achieved was 1.5 h.

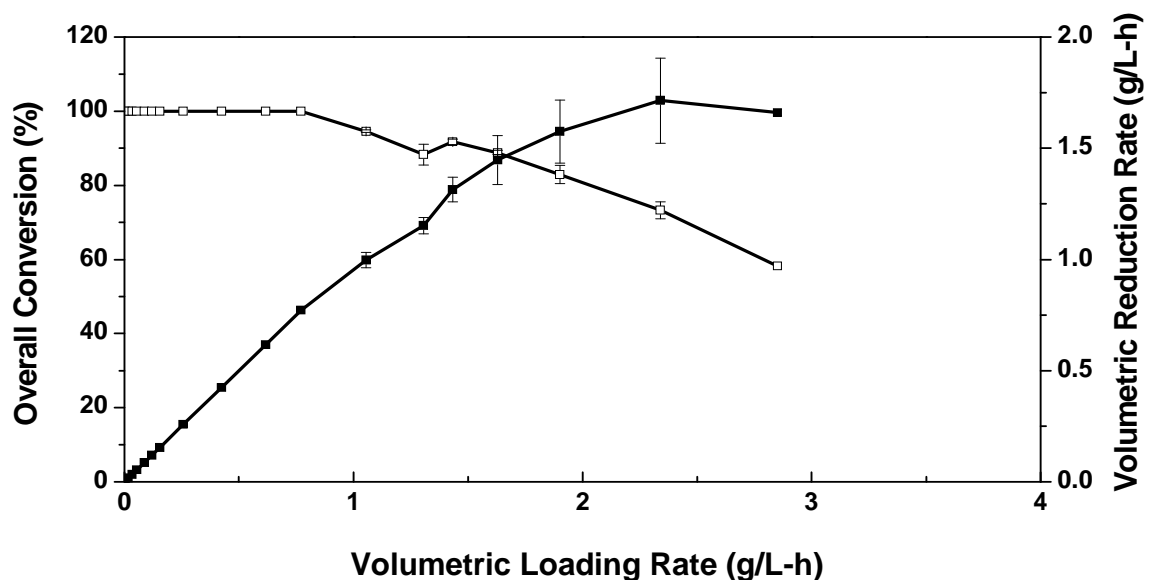


Figure 5.19 Overall conversion and volumetric reduction rate profiles for the bioreactor fed with a medium containing 1.0 g/L of sulphate and packed with sand (□)-Overall conversion profile, (■) – Overall volumetric reduction rate profile.

Similar to volumetric reduction rate profile, the overall lactate utilisation rate and the acetate production rate profiles (Figure 5.20) increased linearly with the increase in the applied volumetric loading rate. The maximum overall lactate utilisation rate and acetate production rate were 3.0 and 2.0 g/L-h, observed at a volumetric loading rate of 2.34 g/L-h. The highest volumetric reduction rate was also observed at this volumetric loading rate. Further increase in volumetric loading rate led to a decline in the overall lactate utilisation and acetate production rates, with the values of 1.81 and 1.19 g/L-h were observed for lactate utilisation and acetate production rates at the highest applied volumetric loading rate of 2.85 g/L-h.

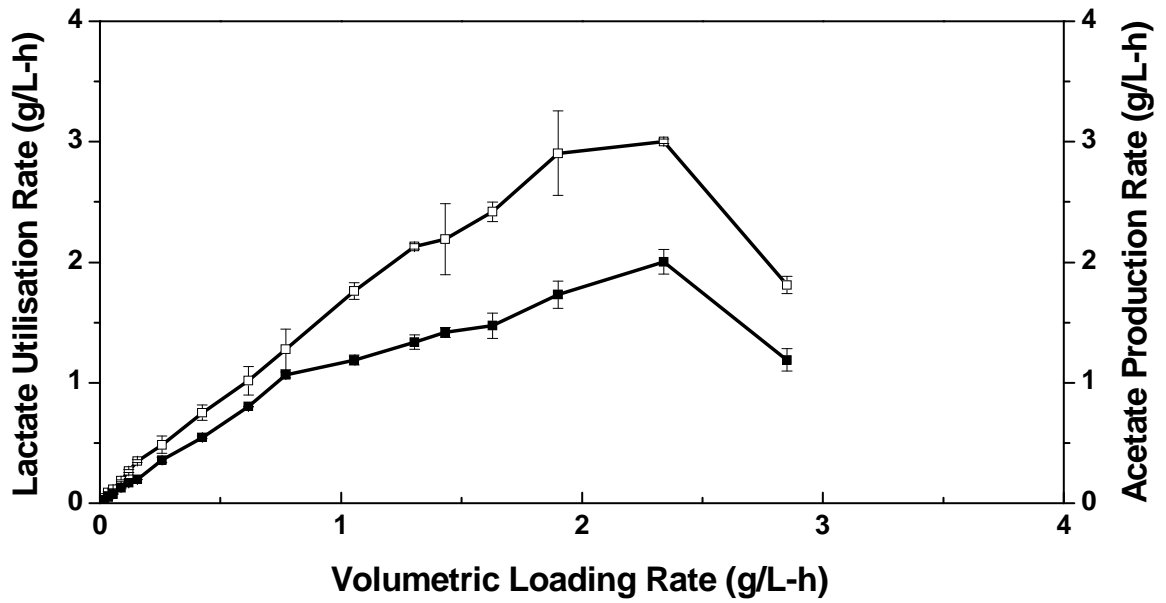


Figure 5.20 Profiles for overall lactate utilisation and acetate production rates for the bioreactor fed with a medium containing 1.0 g/L of sulphate and packed with sand (□) - Overall lactate utilisation rate profile and (■) – Overall acetate production rate profile.

5.1.4. Comparison of Bioreactor Performance with Different Carrier Matrices

The effect of carrier matrix on the performance of the bioreactors will be compared and discussed in the following section. This includes a comparison of residual sulphate concentration, conversions achieved, observed organic acids concentrations and more importantly on the overall volumetric reduction rate achieved in the bioreactors.

The residual sulphate and produced sulphide concentrations and conversion followed the same pattern in all three bioreactors. The increase in volumetric loading rate (decrease in residence time) led to a decrease in conversion, observed as an increase in residual sulphate concentration and a decrease in produced sulphide concentration. The maximum volumetric loading rate (minimum residence time) at which the complete conversion of sulphate (100%) was achieved, however, was dependent on the carrier matrix employed in the bioreactor, with the values for the bioreactors packed with the sand, foam BSP and glass bead were 0.77 g/L-h (residence time: 1.5 h), 0.063 g/L-h (residence time: 17.8 h) and 0.044 g/L-h (residence time: 28.6 h), respectively. The highest produced sulphide concentration was almost the same in all three bioreactors and the observed values were 0.568 g/L, 0.565 g/L and 0.577 g/L for the bioreactors employing sand, foam BSP and glass bead, respectively.

Figure 5.21 compares the overall volumetric reduction rates as a function of volumetric loading rate for the bioreactors operated with different carrier matrices. As can be seen in Figure 5.21, the profiles representing the dependency of volumetric reduction rate on volumetric loading rate coincided with each other. In all cases, the volumetric reduction rate increased linearly as the volumetric loading rate was increased up to a certain level, and then decreased with further increase of volumetric loading rate.

However, the onset of decrease in volumetric reduction rate and the maximum value of volumetric reduction rate were dependent on the carrier matrix employed in the bioreactor.

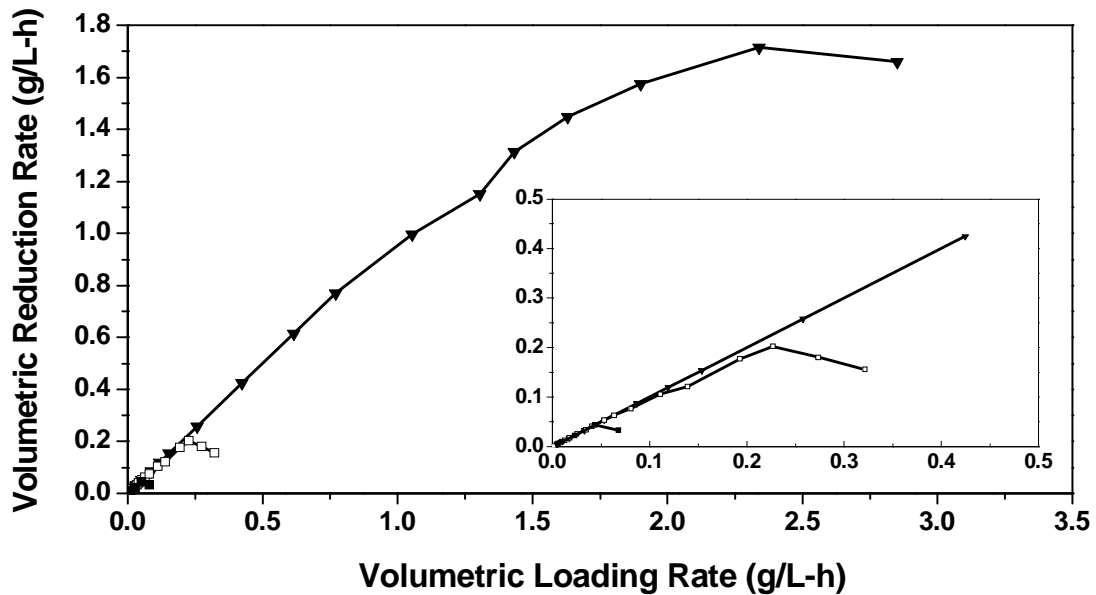


Figure 5.21 Comparison of overall volumetric reduction rate profiles for the bioreactors fed with a medium containing 1.0 g/L of sulphate and packed with different carrier matrices (■)-Glass bead, (□)-Foam BSP and (▼)-Sand bioreactors.

The maximum volumetric reduction rate achieved in each bioreactor and the associated parameters based on the bioreactor void volume are summarized in Table 5.2. A comparison of the data presented in Table 5.2 indicates that the bioreactor packed with glass beads displayed the poorest performance, in which the lowest maximum volumetric reduction rate of 0.04 g/L-h was achieved at the longest residence time of 28.6 h. In the bioreactor packed with foam BSP the maximum volumetric reduction rate of 0.2 g/L-h was obtained at the residence time of 5.3 h which was far better than the

one packed with glass bead. The superior performance with respect to anaerobic reduction of sulphate was achieved in the bioreactor packed with sand in which the highest maximum volumetric reduction rate of 1.7 g/L-h (40 and 8 folds higher than the maximum reduction rates in bioreactors packed with glass beads and foam BSP, respectively) was achieved at the shortest residence time of 0.5 h.

Table 5.2 Maximum volumetric reduction rate and the associated parameters calculated based on void volume

Carrier matrix	Maximum reduction rate achieved (g/L-h)	Loading rate (g/L-h)	Conversion (%)	Residence time (h)	Dilution rate (h⁻¹)
Glass Bead	0.04	0.04	100	28.6	0.04
Foam BSP	0.2	0.22	89	5.3	0.19
Sand	1.71	2.34	73	0.5	2.0

A similar pattern was observed when the total volume of the bioreactor (450 mL) was used for calculation of the volumetric loading and volumetric reduction rates (Table 5.3).

Table 5.3 Maximum volumetric reduction rate and the associated parameters calculated based on the total volume

Carrier matrix	Maximum reduction rate achieved (g/L-h)	Loading rate (g/L-h)	Conversion (%)	Residence time (h)
Glass Bead	0.01	0.01	100	117.2
Foam BSP	0.102	0.112	89	10.5
Sand	0.228	0.312	73	4.0

One of the important parameters influencing the volumetric reduction rate in an immobilized cell bioreactor is the extent of biomass hold-up which can be defined as the quantity of immobilized cells, plus any freely suspended cells which are present in the system. Contribution of freely suspended cells is significant when low volumetric loading rates (dilution rates below the maximum specific growth rate) are applied, while at high volumetric loading rates (dilution rates above the maximum specific growth rate) the freely suspended cells are washed out from the system and the observed volumetric reduction rates are mainly due to activity of immobilized cells. In the case of bioreactor packed with glass beads the maximum volumetric reduction rate was achieved at a dilution rate of 0.035 h^{-1} (Table 5.2) which was below the values of maximum specific growth rate (0.058 to 0.065 h^{-1}) reported for SRB by Moosa et al. (2002). Because of this reason, freely suspended cells must have had a significant contribution in reduction of sulphate to sulphide. By contrast the dilution rates at which maximum volumetric reduction rate was achieved in the bioreactors packed with foam BSP (0.19 h^{-1}) and sand

(2.0 h^{-1}) were significantly higher than the critical values, indicating that the observed volumetric reduction rates were mainly due to activity of immobilized cells. In case of passively immobilized cells, the extent of biomass hold-up determined mainly by the total surface area available for cell immobilization, although properties of the carrier matrix such as electrical charges and pore size could contribute to extent of biomass hold-up (Webb and Dervakos, 1996). As such one of the main contributing factors in superior performances observed in the bioreactor packed with sand (higher volumetric reduction rates at shorter residence times) is the extended surface area which the employment of sand provided for cell immobilization (Table 5.1). Provision of a large surface area for attachment of biomass and formation of biofilm, in fact, could have resulted in higher biomass hold-up in the bioreactor which allowed the efficient conversion of sulphate at much higher volumetric loading rates (shorter residence times) thus leading to enhanced volumetric reduction rates.

An important point which should be considered is that the measurement of surface area by the available surface analyzer provides information accurate enough to compare the surface area of three different carrier matrices and to establish a trend. The absolute values for specific surface area of each carrier matrix should be treated cautiously since analytical error in measurement of surface area for materials having relatively low surface area is significant.

The coupling of lactate utilization with the sulphate reduction was observed in all three bioreactors. As the volumetric loading rate was increased, the residual lactate concentrations increased, with corresponding decrease in the produced acetate concentration. The highest lactate utilisation rate for the bioreactors packed with sand, foam BSP and glass bead were 3.0, 0.27 and 0.08 g/L-h, respectively. The highest

acetate production rates observed in the bioreactors packed with sand, foam BSP and glass bead were 2.0, 0.17 and 0.04 g/L-h, respectively.

The results obtained in this part of the work indicate that the kinetics of sulphate reduction in an immobilized cell system depends highly on the employed carrier matrix, specifically the total surface area which matrix provides for the establishment of the biofilm. The bioreactor packed with the sand clearly showed a superior performance, and as such sand was used as a carrier matrix to evaluate the effect of initial sulphate concentration on the kinetics of sulphate reduction.

5.2. Effects of Sulphate Concentration

After completing the experiments with different carrier matrices, the effects of initial sulphate concentration and volumetric loading rate on the kinetics of sulphate reduction were studied by conducting two additional experimental runs, using media containing 2.5 and 5.0 g/L of sulphate and sand as a carrier matrix. The following sections discuss the performance of bioreactors operating with feed sulphate concentration of 2.5 and 5.0 g/L. A comparison of the kinetic data obtained with various feed sulphate concentrations will be presented.

5.2.1. Performance of Bioreactor –Feed Sulphate Concentration of 2.5 g/L

Figure 5.22 shows the stepwise increase in the feed flow rate as a function of time. The initial flow rate employed in this experiment was 0.28 mL/h and it was

increased stepwise to a maximum flow rate of 30.12 mL/h. The bioreactor was run for a period of 151 days. The pH of the effluent from the bioreactor varied between 7.7- 8.8.

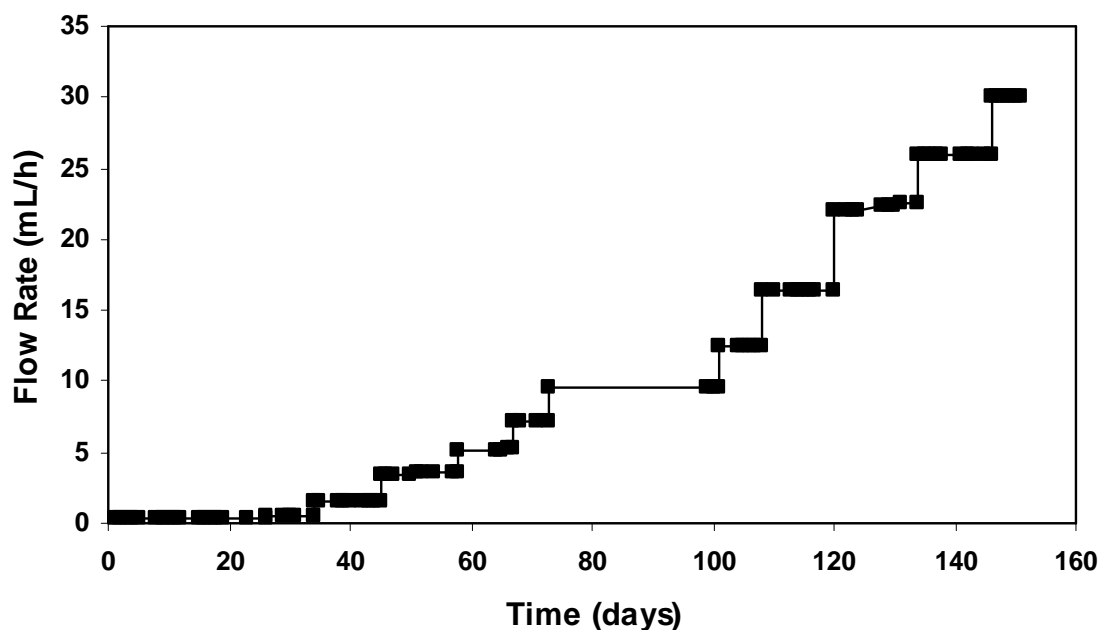


Figure 5.22 Variation of feed flow rate as a function of time for the bioreactor fed with a medium containing 2.5 g/L of sulphate and packed with sand

5.2.1.1. Protein Concentration Profiles

Figure 5.23 represents the liquid phase protein concentration profiles for the bioreactor operating with the feed sulphate concentration of 2.5 g/L. The protein concentration in the lower part of the bioreactor was found to be higher when compared with the middle part which in turn was higher than the upper part of the bioreactor. The protein concentrations in the liquid phase increased with an increase in volumetric loading rate up to 1.024, 0.742 and 0.435 g/L-h in the lower, middle and upper parts of

the bioreactor, respectively. This initial increase in the liquid phase protein concentration may be due to availability of more substrate at increased volumetric loading rates. The maximum protein concentrations observed at the volumetric loading rates of 1.024, 0.742 and 0.435 g/L-h were 69.1, 49.6 and 26.3 mg/L in the lower, middle and upper parts of the bioreactor, respectively. Applying higher volumetric loading rates reduced the protein concentration sharply, especially in the lower and middle parts of the bioreactor, which could be explained by the washing out of the freely suspended cells by the effluent stream at a rate faster than cell reproduction rate. In the upper part, after attaining the highest protein concentration at a volumetric loading rate of 0.435 g/L-h, a gradual decrease in the protein concentration was observed with the increase in volumetric loading rates. The observed protein concentration at the highest applied volumetric loading rate of 1.32 g/L-h was 32.32, 14.2 and 8.716 mg/L at the lower, middle and upper parts of the bioreactor, respectively.

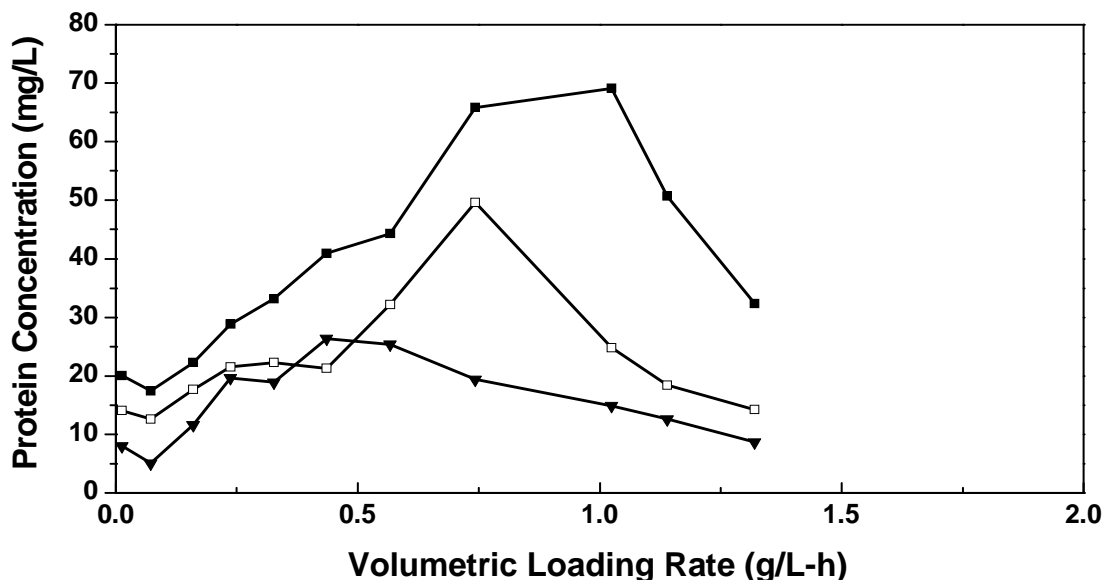


Figure 5.23 Liquid phase protein concentration profile for the bioreactor fed with a medium containing 2.5 g/L of sulphate and packed with sand (■)-Lower, (□)-Middle and (▼)-Upper parts.

5.2.1.2. Residual Sulphate and Produced Sulphide Concentration and Conversion

Profiles

Figure 5.24 represents the profiles for the residual sulphate and the produced sulphide concentrations for the three different regions of the bioreactor. Figure 5.25 shows the conversion profile as a function of volumetric loading rates. Complete conversion (residual sulphate concentration of zero) was never achieved in the lower part of the bioreactor even at the initial volumetric loading rate of 0.012 g/L-h (residence time: 224.7 h). Conversion in the lower part was observed to vary from 98 to 86.5% for volumetric loading rates up to 0.237 g/L-h (residence time: 11.4 h) with the lower values

were noticed at higher volumetric loading rates. Further increase in the volumetric loading rate led to sharp drop in conversion in the lower part of the bioreactor. In the middle and upper parts of the bioreactor the residual sulphate concentration remained zero for volumetric loading rates in the range 0.027 to 0.072 g/L-h (residence time: 102.8 to 37.3 h). Conversions in the range 100 – 90% was observed up to a volumetric loading rate of 0.567 g/L-h (residence time: 4. 8 h) in the middle part of the bioreactor. In the upper part of the bioreactor, high conversion values of 100 - 90.4% was observed up to volumetric loading rate of 0.742 g/L-h (residence time: 3.7 h). A sharp decrease in the conversion was seen in all the three parts of the bioreactor with the further increase in the volumetric loading rate. With the highest applied volumetric loading rate of 1.32 g/L-h (residence time: 2.0 h), the residual sulphate concentration was 2.17 g/L (17.3% conversion), 1.47 g/L (43.8% conversion) and 1.225 g/L (53.4% conversion) in the lower, middle and upper parts of bioreactor, respectively. The maximum produced sulphide concentration observed at a volumetric loading rate of 0.012 g/L-h was 0.95 g/L, 1.02 g/L and 1.06 g/L in the lower, middle and upper parts of the bioreactor, respectively.

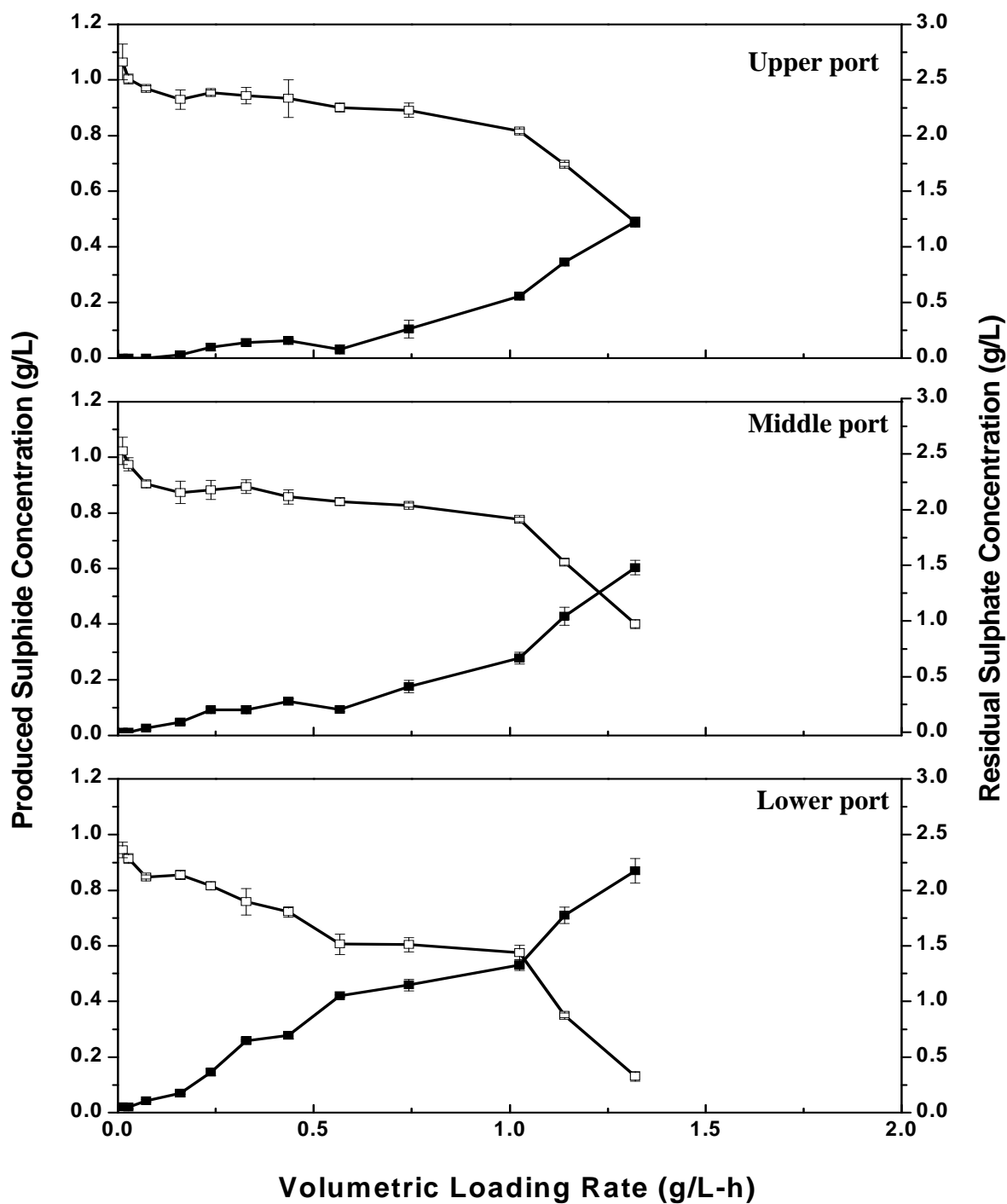


Figure 5.24 Residual sulphate and produced sulphide concentration profiles for the bioreactor fed with a medium containing 2.5 g/L of sulphate and packed with sand (□)-Produced sulphide concentration profile and (■) – Residual sulphate concentration profile.

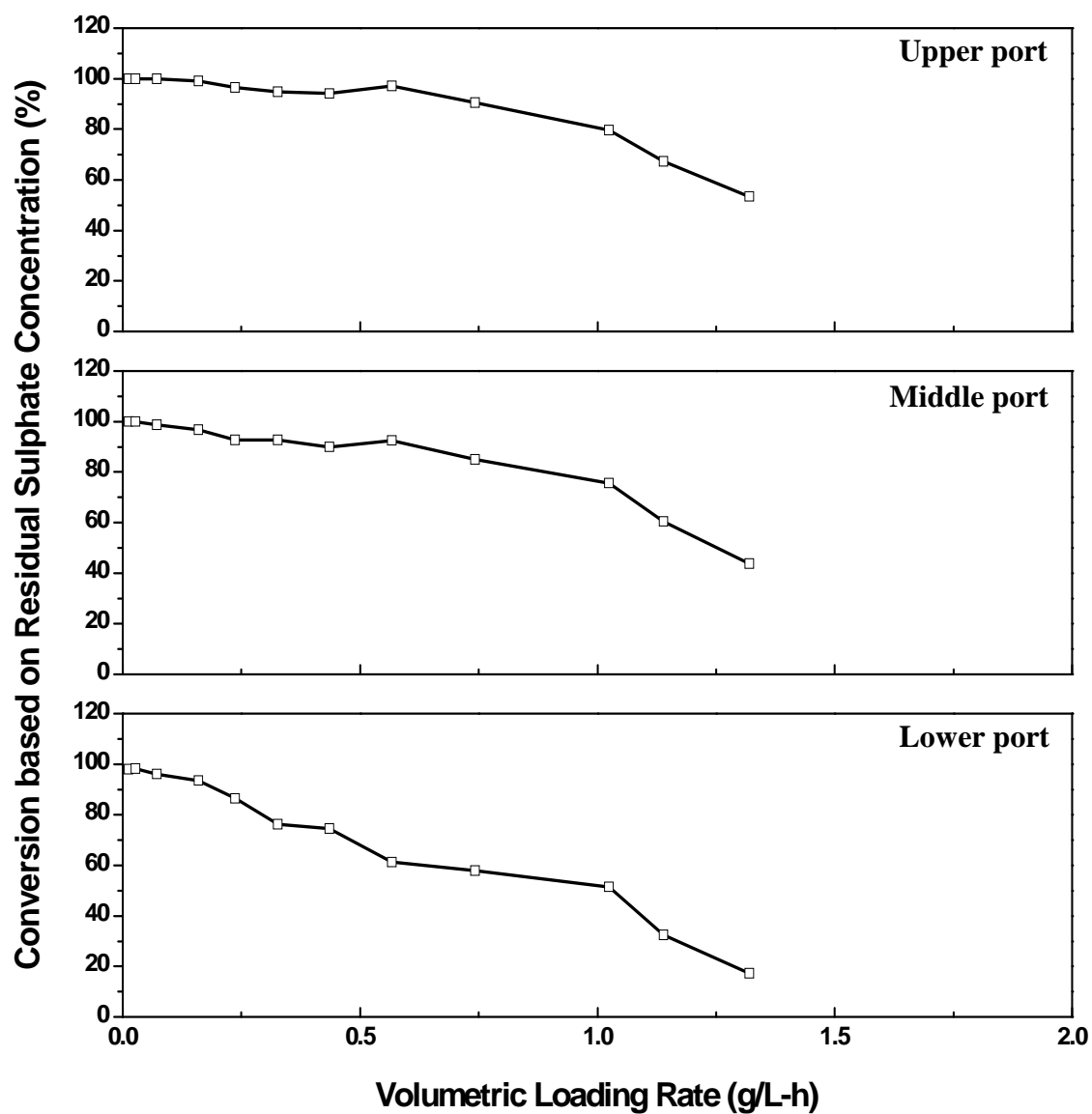


Figure 5.25 Conversion profile calculated based on sulphate concentration for the bioreactor fed with a medium containing 2.5 g/L of sulphate and packed with sand

5.2.1.3. Organic Acids (*Lactate and Acetate*) Concentration Profiles

The lactate and acetate concentration profiles are shown in Figure 5.26. The coupling of lactate utilization on sulphate reduction was also seen in this experimental run. The residual lactate concentration increased with increase in volumetric loading rate, while acetate production in the bioreactor decreased. From Figure 5.26 it can be seen that the residual lactate concentration remained always above zero. At a constant applied volumetric loading rate the lactate concentration, similar to the residual sulphate concentration decreased along the length of the bioreactor. In the lower port, up to a volumetric loading rate of 0.327 g/L-h (conversions of 98 to 76%) the lactate concentration was in the range 2.8 to 3.4 g/L. In the middle part of the bioreactor for volumetric loading rates up to 0.567 g/L-h (conversions of 100 to 90%), the lactate concentration was in the range of 2.3 to 2.8 g/L. Residual lactate concentration ranging from 1.5 to 2.8 g/L was observed in the upper part of the bioreactor for volumetric loading rates up to 0.74 g/L-h (conversions of 100-90%). When the volumetric loading rate was increased further, a steep increase in the residual lactate concentration was observed in all the three parts of the bioreactor. At the highest applied volumetric loading rate of 1.32 g/L-h, the residual lactate concentration was found to be 7.57, 6.19 and 5.55 g/L in the lower, middle and upper ports of the bioreactor, respectively. The highest acetate concentration for the lower, middle and upper parts of the bioreactor was 2.3, 2.8 and 3.2 g/L, respectively and observed at the initial stages of the bioreactor operation.

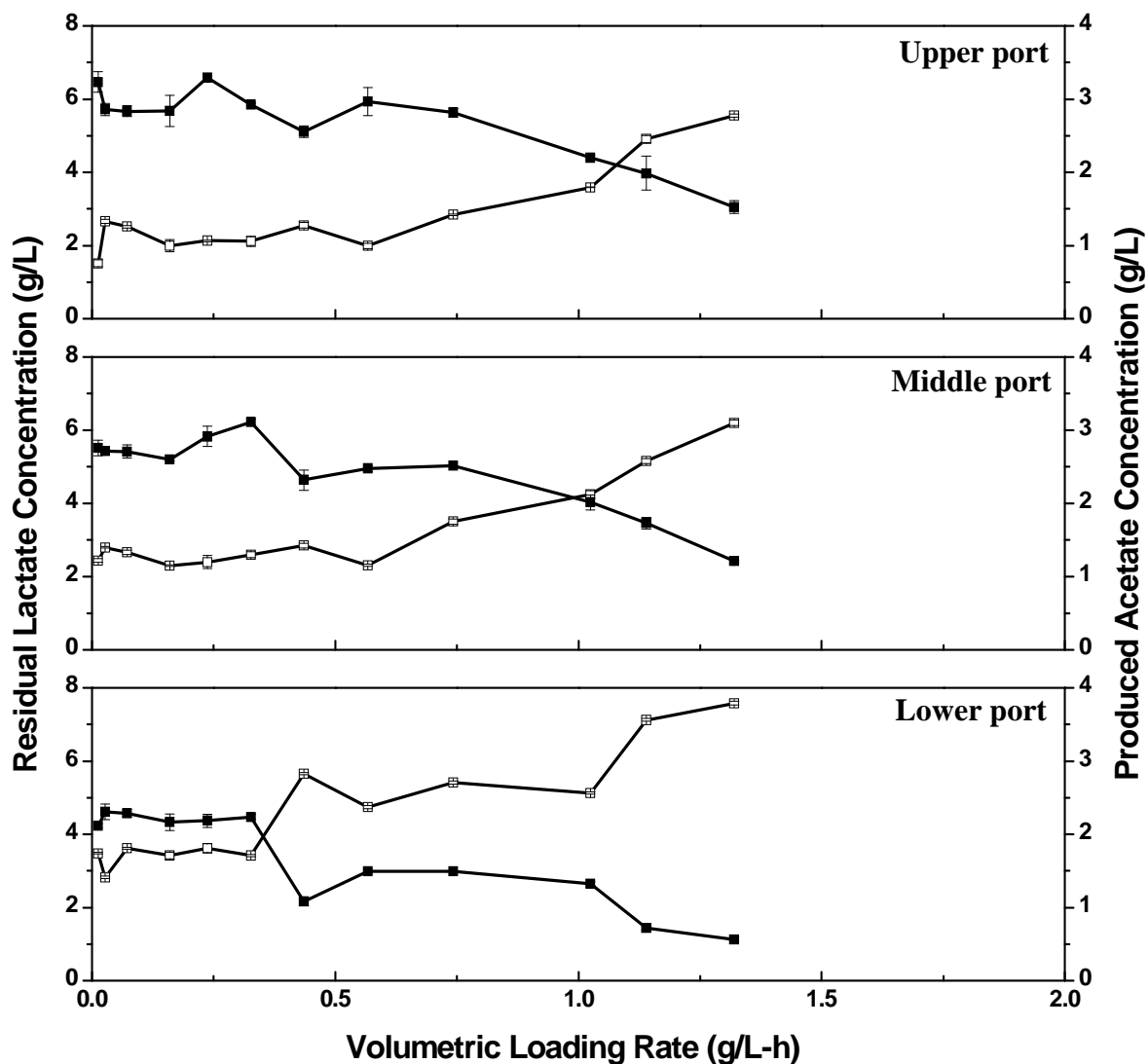


Figure 5.26 Residual lactate and produced acetate concentration profiles for the bioreactor fed with a medium containing 2.5 g/L of sulphate and packed with sand (□)- Residual lactate concentration profile and (■) – Produced acetate concentration profile.

5.2.1.4. Volumetric Reduction Rate, Lactate Utilization and Acetate Production Rates

The overall conversion and volumetric reduction rate are shown in Figure 5.27. The overall volumetric reduction rate was found to increase linearly with increases in the volumetric loading rate. The overall conversion was high (100 - 90%) up to a volumetric loading rate of 0.74 g/L-h (residence time: 3.7 h). The maximum overall volumetric reduction rate was 0.81 g/L-h, observed at a volumetric loading rate of 1.02 g/L-h (residence time: 2.7 h), with a corresponding conversion of 79.6%. When the residence time was decreased below 2.66 h, a drop in volumetric reduction rate was observed. At the highest applied volumetric loading rate of 1.32 g/L-h (residence time: 2.0 h), the overall volumetric reduction rate and conversion were dropped to 0.7 g/L-h and 53%, respectively. The lowest residence time for which complete conversion of sulphate to sulphide achieved was 37.3 h.

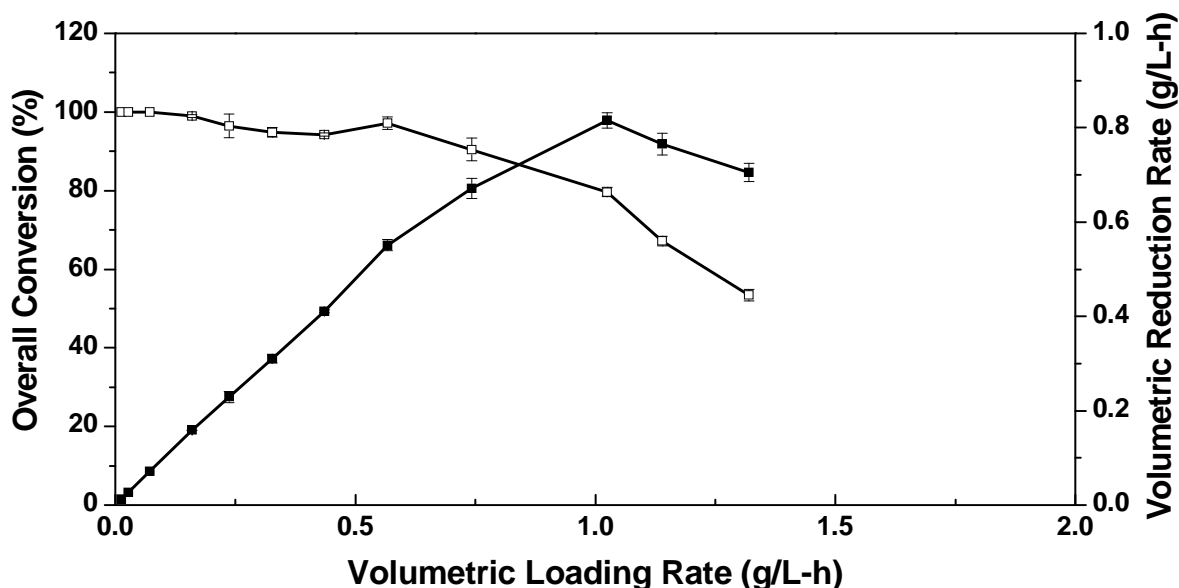


Figure 5.27 Overall conversion and volumetric reduction rate profiles for the bioreactor fed with a medium containing 2.5 g/L of sulphate and packed with sand (□)-Overall conversion profile and (■) – Overall volumetric reduction rate profile.

Similar to overall volumetric reduction rate profile, the overall lactate utilisation rate and the acetate production rate (Figure 5.28) increased with the increase in the applied volumetric loading rate up to a certain value. The maximum overall lactate utilisation rate and acetate production rate were 1.12 and 0.82 g/L-h, observed at a volumetric loading rate of 1.02 g/L-h. The highest volumetric reduction rate was also observed at this volumetric loading rate. Further increase in volumetric loading rate led to a decrease in the overall lactate utilisation and acetate production rates with their values being 0.52 and 0.38 g/L-h at the highest applied volumetric loading rate of 1.32 g/L-h.

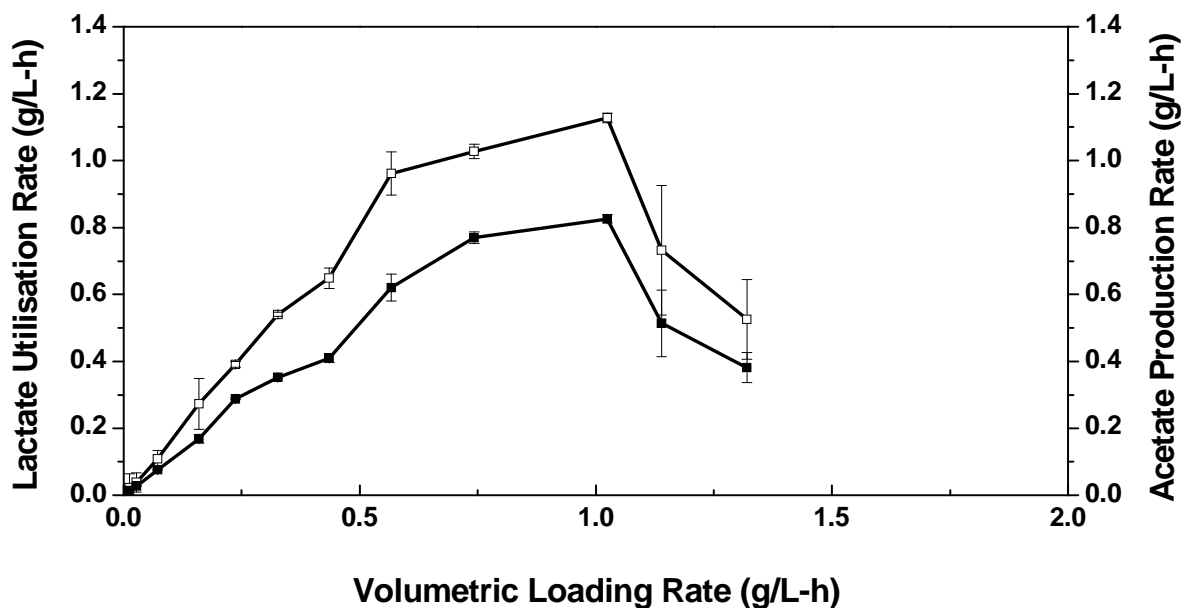


Figure 5.28 Overall lactate utilisation and acetate production rate profiles for the bioreactor fed with a medium containing 2.5 g/L of sulphate and packed with sand (□) - Overall lactate utilisation rate profile and (■) – Overall acetate production rate profile.

5.2.2. Performance of Bioreactor –Feed Sulphate Concentration of 5.0 g/L

The flow rate applied in this experimental run ranged from 0.3 to 41.2 mL/h as shown in Figure 5.30. The total time required to complete this experimental run was 224 days. Compared with the other two experimental runs, in this set of experiment the establishment of steady-state conditions at each flow rate needed a longer time. Like other experimental runs, this bioreactor was operated until a decrease in volumetric reduction rate was observed.

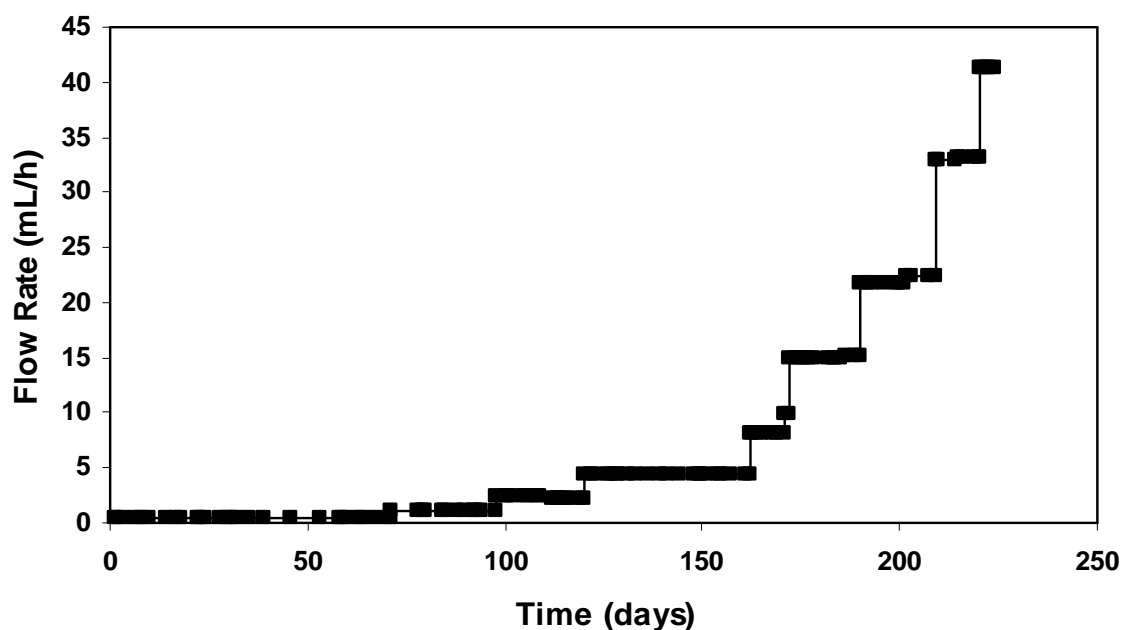


Figure 5.29 Variation of feed flow rate as a function of time for the bioreactor fed with a medium containing 5.0 g/L of sulphate and packed with sand

5.2.2.1. Protein Concentration Profile

The protein concentration profiles in the different regions of the bioreactor are shown in Figure 5.30. At a constant volumetric loading rate the protein concentration was found to decrease towards the upper part of the bioreactor. A sharp increase in the protein concentration was noticed up to a volumetric loading rate of 0.094 g/L-h, which corresponds to a residence time of 54.4 h in all parts of the bioreactor. Increase in volumetric loading rates beyond this point led to a gradual increase in the protein concentration in all parts of the bioreactor until it reached the highest value. In the lower port the highest liquid phase protein concentration of 46.85 mg/L was measured at a volumetric loading rate of 0.69 g/L-h, corresponding to a residence time of 7.4 h. In the

upper port the biomass concentration reached the maximum value of 32.44 mg/L at the same volumetric loading rate, while the highest value of 35.7 mg/L was attained at a volumetric loading rate of 0.367 g/L-h in the middle port. Further increase in the volumetric loading rate decreased the liquid phase protein concentration. At the highest applied volumetric loading rate (3.52 g/L-h), the liquid phase protein concentrations were 21.5, 10.9 and 3.7 mg/L in the lower, middle and upper parts of the bioreactor, respectively.

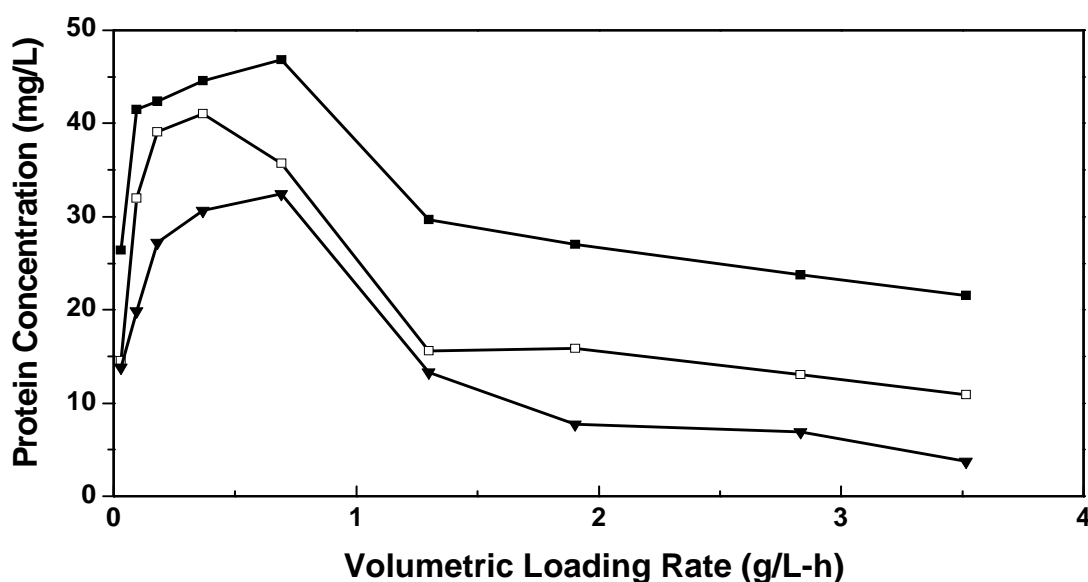


Figure 5.30 Liquid phase protein concentration profiles for the bioreactor fed with a medium containing 5.0 g/L of sulphate and packed with sand (■)-Lower, (◊)-Middle and (▼)-Upper parts.

5.2.2.2. Residual Sulphate and Produced Sulphide Concentrations and Conversion Profiles

Following the inoculation with the SRB culture, the bioreactor was run batch wise to achieve 100% conversion. Despite running the bioreactor for 31 days in the batch mode, the highest conversions achieved were 74, 93 and 90% in the lower, middle and upper parts of the bioreactor, respectively. After the establishment of steady state conditions, the bioreactor was switched to continuous mode. The residual sulphate and the produced sulphide concentration profiles for three regions of bioreactor are shown in Figure 5.31. The conversion profile is presented in Figure 5.32. The residual sulphate concentration never reached zero (100% conversion was not achieved) in any part of the reactor. The lowest residual sulphate concentration observed was 1.08 g/L (conversion: 79%), 0.86 g/L (conversion: 83.2%) and 0.52 g/L (conversion: 89.7%) in the lower, middle and upper ports of the bioreactor, respectively, attained at a volumetric loading rate of 0.094 g/L-h (residence time: 54.4 h). The residual sulphate concentration was almost constant in all three parts of the bioreactor for volumetric loading rates up to 0.179 g/L-h, which corresponds to a residence time of 28.4 h. Gradual increase in the volumetric loading rate above 0.179 g/L-h led to a linear increase in the residual sulphate concentration in all the three ports of bioreactor. The difference in the residual sulphate concentration between lower, middle and upper ports was low. This indicated that the microbial activity was not very significant in the middle and upper parts of the bioreactor. The reason for the observed pattern could be possibly attributed to inhibitory effect of high concentration of sulphide produced in the lower part of the bioreactor which hampered the activity of SRB in the middle and upper parts of the bioreactor. The highest sulphide concentration measured during the batch operation was 1.33 g/L, 1.54

g/L and 1.59 g/L in the lower, middle and upper parts of the bioreactor, respectively. During continuous operation the produced sulphide concentrations were 1.21 g/L, 1.3 g/L and 1.36 in the lower, middle and upper parts of the bioreactor, respectively. At the highest applied volumetric loading rate of 3.52 g/L-h (residence time: 1.46 h), the residual sulphate concentration was 4.87 g/L (conversion: 4.8%), 4.74 g/L (conversion: 7.4%) and 4.59 g/L (conversion: 10.2%) in the lower, middle and upper parts of the bioreactor, respectively.

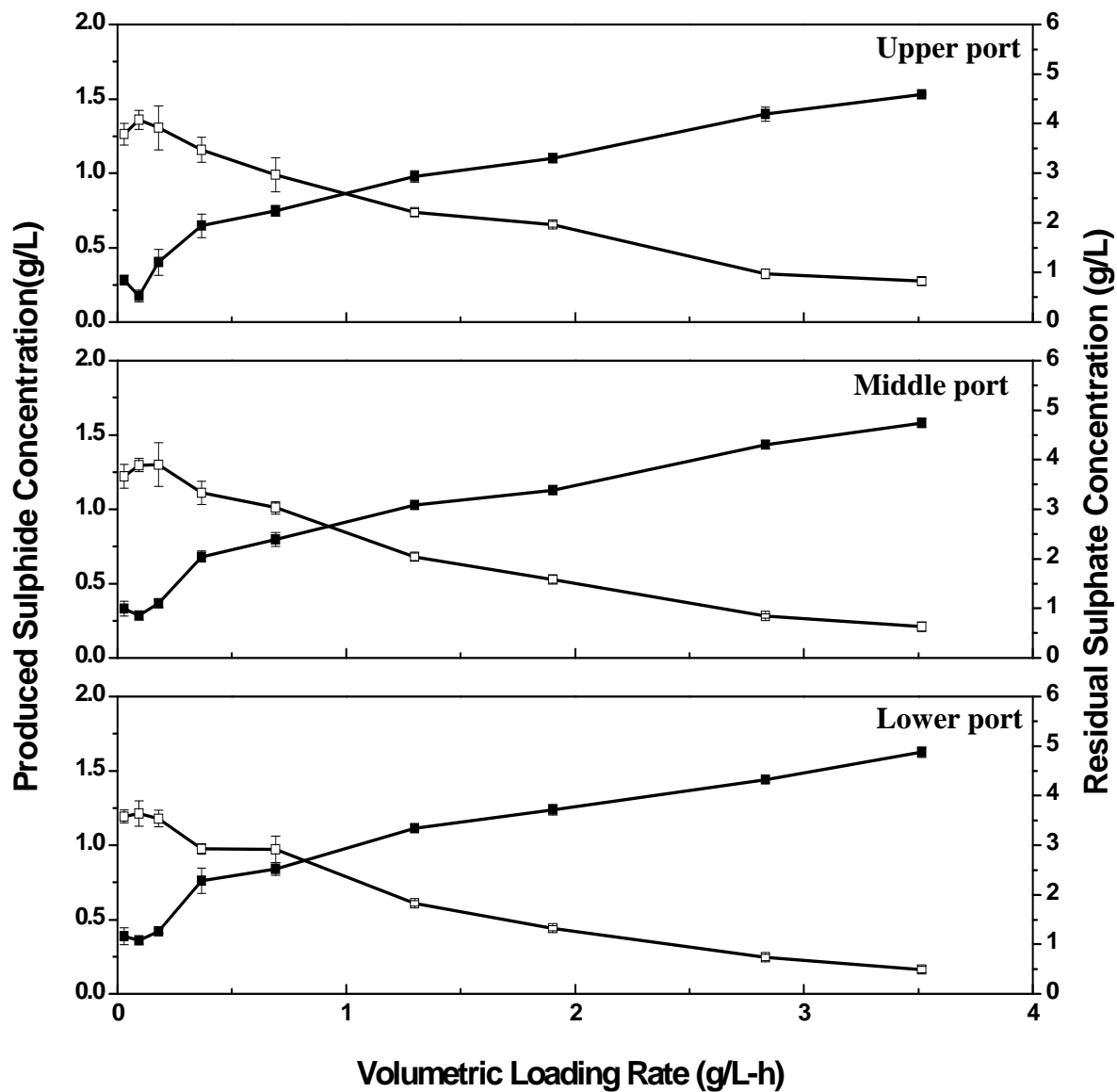


Figure 5.31 Residual sulphate and produced sulphide concentration profiles for the bioreactor fed with a medium containing 5.0 g/L of sulphate and packed with sand (□)-Produced sulphide concentration profile and (■) – Residual sulphate concentration profile.

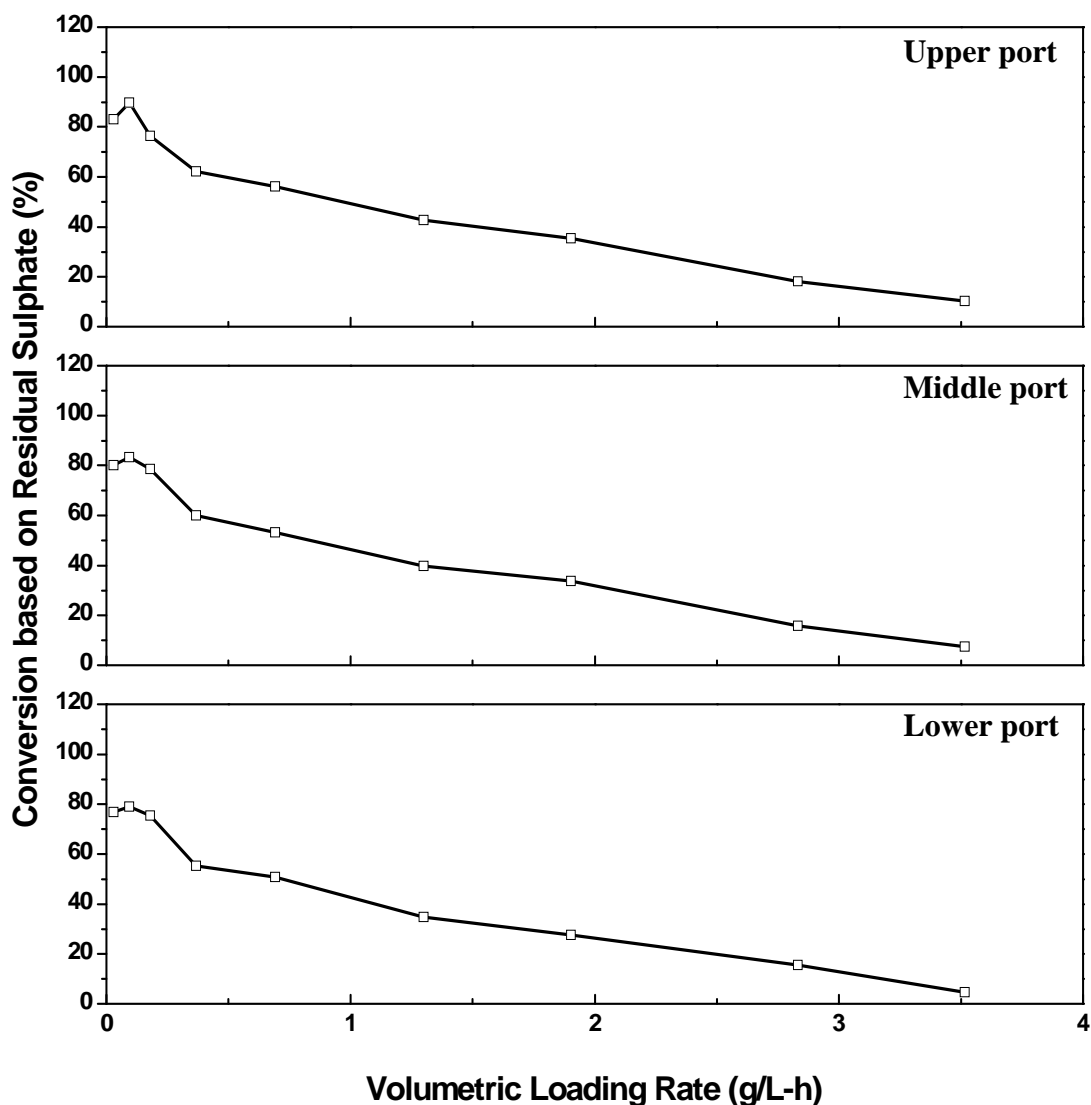


Figure 5.32 Conversion profiles based on sulphate concentration for the bioreactor fed with a medium containing 5.0 g/L of sulphate and packed with sand

5.2.2.3. Organic Acid (Lactate and Acetate) Concentration Profiles

Figure 5.33 shows the residual lactate and produced acetate concentration profiles in different regions of bioreactor. From Figure 5.33 it is clear that the residual

lactate concentration and the produced acetate concentration follow the same pattern as the residual sulphate and the produced sulphide concentration, respectively. Up to the volumetric loading rate of 0.69 g/L-h (residence time: 7.4 h) a sharp increase in the residual lactate concentration was observed in all the three ports of the bioreactor. Further increase in the volumetric loading rate led to the gradual increase in the residual lactate concentration until the highest applied volumetric loading rate. As observed in the bioreactor operated with 2.5 g/L feed sulphate concentration, the residual lactate concentration never reached zero. The lowest residual lactate concentration was 2.85 g/L observed in the upper part of the bioreactor and at the lowest applied volumetric loading rate of 0.028 g/L-h. The maximum produced acetate concentration observed was 7.17 g/L, achieved at the upper part of the bioreactor.

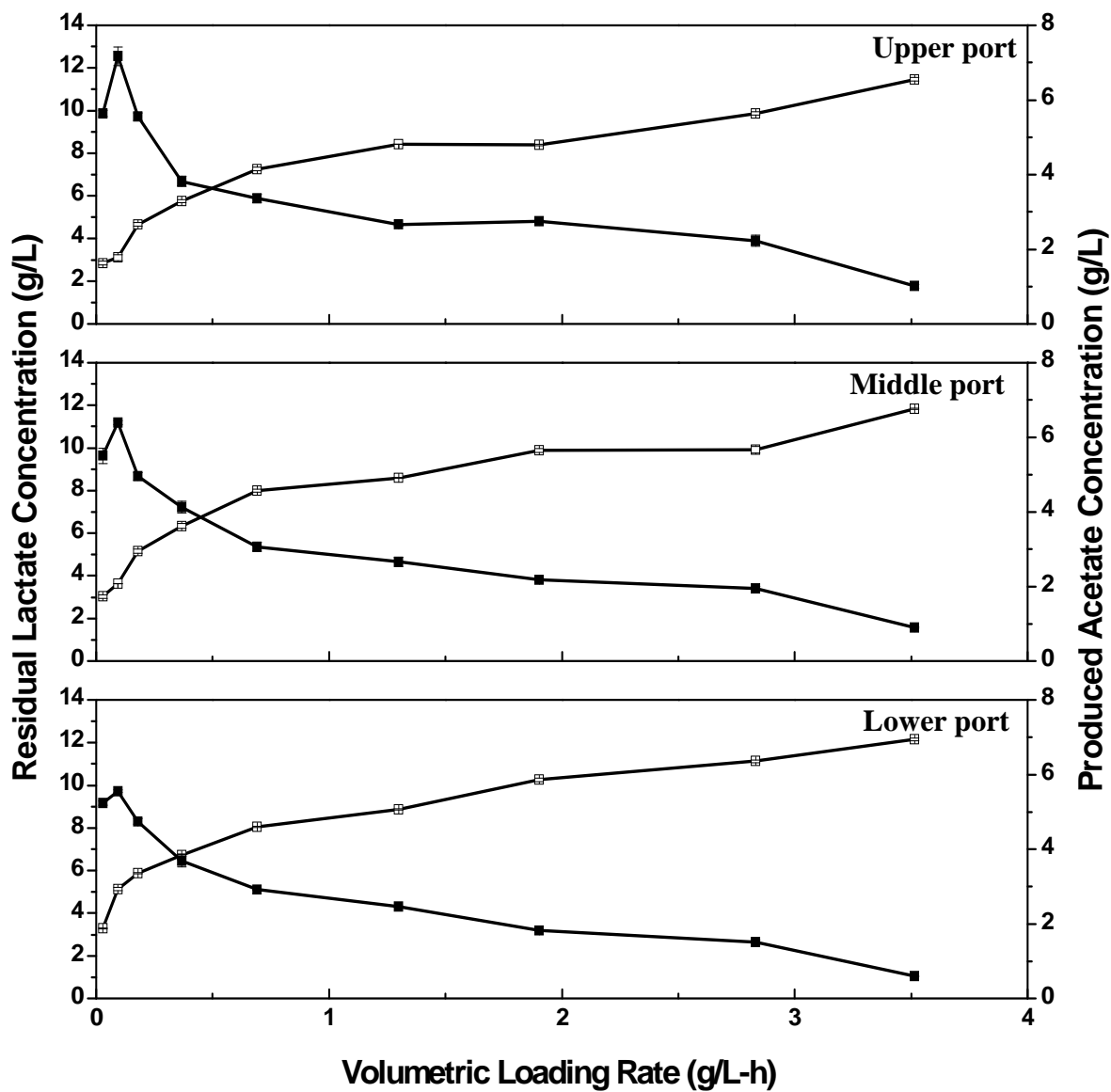


Figure 5.33 Residual lactate and produced acetate concentration profiles for the bioreactor fed with a medium containing 5.0 g/L of sulphate and packed with sand
 (□)- Residual lactate concentration profile, (■) – Produced acetate concentration profile.

5.2.2.4. Volumetric Reduction Rate, Lactate Utilization and Acetate Production Rates

Figure 5.34 shows the profiles for the overall conversion and volumetric reduction rates. Complete conversion of sulphate to sulphide was never achieved in the entire run. The maximum overall conversion of 89.8% was observed at a volumetric loading rate of 0.094 g/L-h (residence time: 54.4 h). The linear increase in volumetric reduction rate was observed with increased volumetric loading rates up to 1.9 g/L-h (residence time: 2.7 h). The maximum overall volumetric reduction rate achieved at this volumetric loading rate was 0.675 g/L-h, with a corresponding conversion of 35.4%. When the volumetric loading rate was increased above 0.66 g/L-h, the overall volumetric reduction rate was decreased. At the volumetric loading rate of 2.83 g/L-h (residence time: 1.8 h) and 3.52 g/L-h (residence time: 1.5 h), the overall volumetric reduction rate dropped to 0.51 and 0.36 g/L-h, respectively.

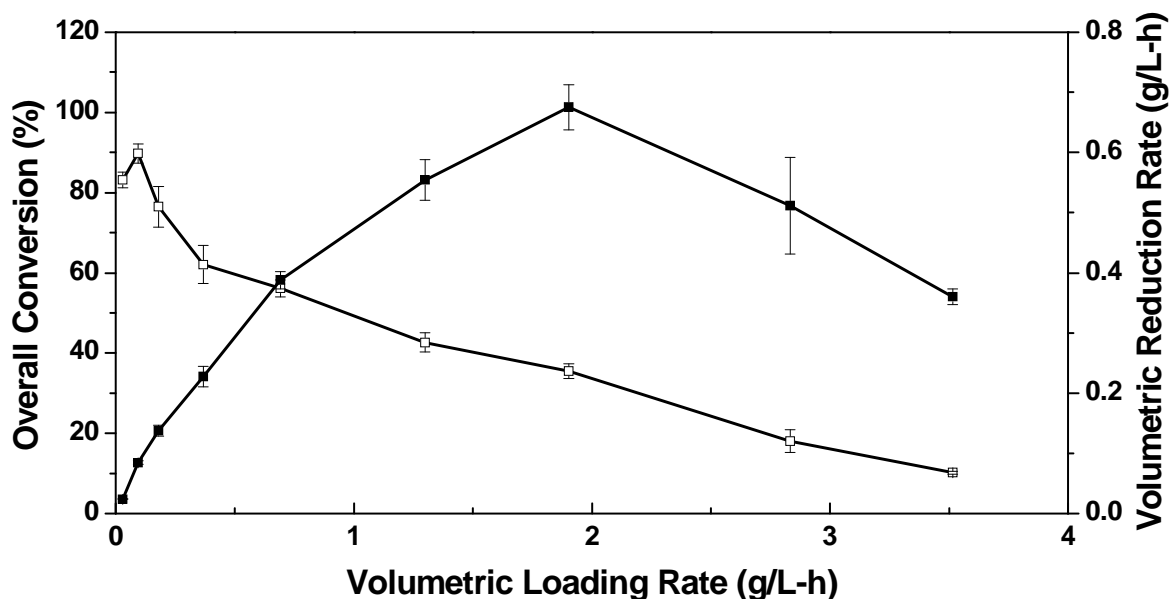


Figure 5.34 Overall conversion and volumetric reduction rate profiles for the bioreactor fed with a medium containing 5.0 g/L of sulphate and packed with sand (□)-Overall conversion profile and (■) – Overall volumetric reduction rate profile.

Figure 5.35 shows the lactate utilisation rate and the acetate production rate profiles. As observed in the previous experimental runs the lactate utilisation rate and the acetate production rate increased with increase in volumetric loading rates up to a certain value. The maximum observed lactate utilisation and acetate production rates were 1.78 and 1.02 g/L-h, respectively at a volumetric loading rate of 1.9 g/L-h (residence time: 2.7 h). Increase in the volumetric loading rate beyond 1.9 g/L-h led to the decrease in both lactate utilisation and the acetate production rates. When the volumetric loading rate was increased to 3.52 g/L-h (highest applied value; residence time of 1.45 h) lactate utilisation and the acetate production rates dropped to 1.19 and 0.69 g/L-h, respectively.

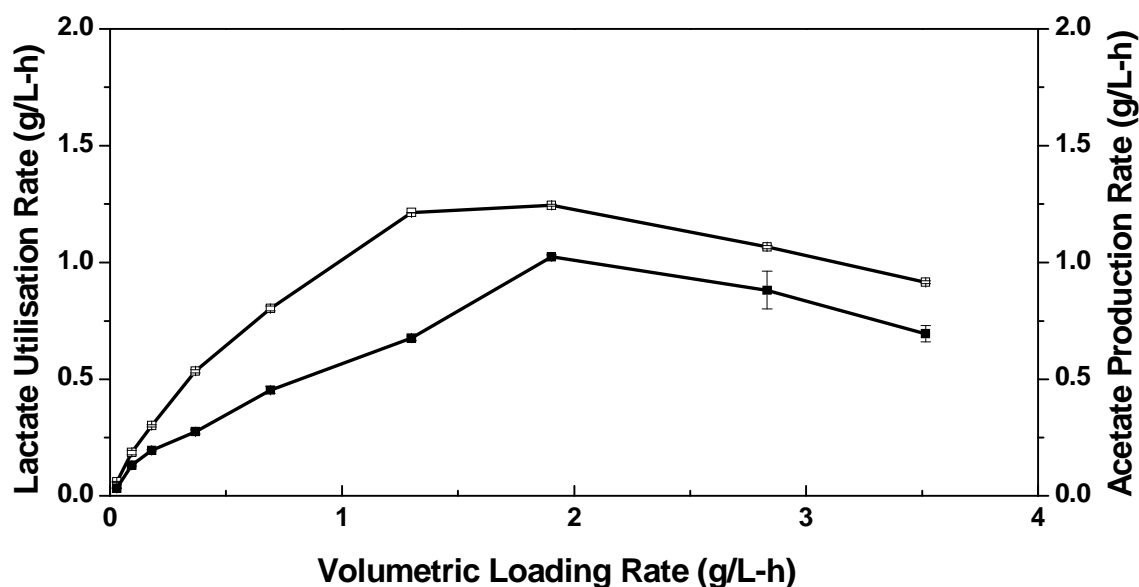


Figure 5.35 Overall lactate utilisation and acetate production rates profile for the bioreactor fed with a medium containing 5.0 g/L of sulphate and packed with sand
 (□) - Overall lactate utilisation rate profile, (■) – Overall acetate production rate profile.

5.2.3. Comparison of the Bioreactor Performance with Media Containing Different Concentrations of Sulphate

The results of the kinetic studies with various feed sulphate concentrations clearly indicate that the initial concentration of sulphate, as well as its volumetric loading rate affected the anaerobic reduction of sulphate in an immobilized cell system. As the initial sulphate concentration was increased from 1.0 to 5.0 g/L, a decrease in the overall performance of the bioreactor (as assessed by the sulphate volumetric reduction rate) was observed. The maximum protein concentration decreased as the initial feed sulphate concentration was increased. The maximum protein concentrations observed

were 140.3, 69.1 and 46.85 mg/L for 1.0, 2.5 and 5.0 g/L feed sulphate concentration, respectively. Complete conversion of sulphate was achieved up to the volumetric loading rate of 0.771 g/L-h (residence time: 1.47 h) and 0.072 g/L-h (residence time: 37.3 h) for feed sulphate concentration of 1.0 and 2.5 g/L, respectively. In the bioreactor operated with 5.0 g/L feed sulphate concentration, complete conversion of sulphate was never achieved, neither during the batch wise operation nor when the lowest volumetric loading rate was applied during the continuous operation. A possible explanation for incomplete conversion of sulphate in this bioreactor could be the inhibitory effects of high sulphide concentrations produced in the bioreactor. The highest produced sulphide concentrations observed in the bioreactors operated with 1.0 and 2.5 g/L of initial feed sulphate concentration were 0.56 g/L and 1.06 g/L, respectively, while in the bioreactor fed with 5.0 g/L sulphate the highest sulphide concentration was 1.35 g/L.

The volumetric reduction rates obtained in the bioreactors fed with media containing 1.0, 2.5 and 5.0 g/L sulphate are compared in Figure 5.36. The dependency of the volumetric reduction rate on volumetric loading rate for bioreactors fed with 2.5 and 5.0 g/L of initial sulphate was similar to that observed with 1.0 g/L sulphate. However, the initial concentration of sulphate in the feed influenced the performance of the bioreactor and significantly lower volumetric reduction rates were observed when concentration of sulphate in the feed was increased from 1.0 to 5.0 g/L. The maximum volumetric reduction rate achieved in the bioreactors operating with the feed sulphate concentration of 1.0, 2.5 and 5.0 g/L were 1.71, 0.82 and 0.68 g/L-h, respectively.

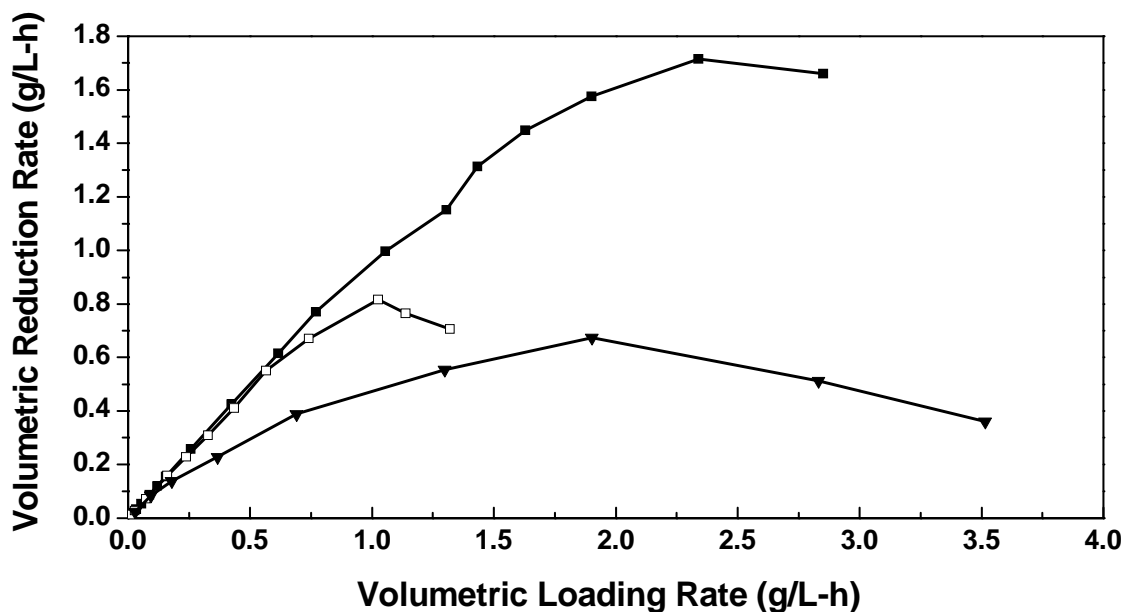


Figure 5.36 Comparison of volumetric reduction rates for the bioreactors fed with media containing different sulphate concentrations and packed with sand (■)-1.0 g/L, (□)-2.5 g/L and (▼)-5.0 g/L of initial sulphate concentration fed bioreactors.

As expected, the initial concentration of sulphate in the feed also influenced the utilisation rate of lactate and production rate of acetate, with highest rates observed in the bioreactor fed with the lowest feed sulphate concentration. The maximum values observed for the lactate utilisation and acetate production rates were 3.0 and 2.0 g/L-h for 1.0 g/L feed sulphate concentration, 1.35 and 0.82 g/L-h for the 2.5 g/L feed sulphate concentration and 1.24 and 1.02 g/L-h for 5.0 g/L feed sulphate concentration, respectively.

Theoretically, incomplete oxidizer SRB need to oxidize two moles of lactate to reduce one mole of sulphate. Oxidation of each mole of lactate results in production of

one mole acetate. Using the experimental values of volumetric reduction rate obtained in the experimental runs and the stoichiometry of sulphate reduction and lactate oxidation, the theoretical lactate utilization and acetate production rates were calculated and compared with the experimental values in parity charts, Figure 5.37 and Figure 5.38. As can be seen, apart from a few data points, the calculated values for lactate utilization and acetate production rates are in close agreement with the values determined experimentally. The differences between the experimental and calculated values can be explained through a number of facts. First, heterotrophic bacteria were a minor component of the mixed culture and could have contributed to the consumption of lactate. Second, in calculating the lactate utilization and acetate production rate it was assumed that only incomplete oxidisers were present. In reality this might not be the case and there is a possibility for existence of the complete oxidiser in the SRB mixed culture.

Figure 5.39 shows the production rate of acetate as a function of lactate utilization rate, based on the experimental data obtained in the bioreactors packed with sand and fed with media containing 1, 2.5 and 5 g/L of sulphate. Fitting a line through the whole range of data, the value of yield was determined to be 0.66 g acetate ion/g lactate ion (Regression coefficient: 0.97). Based on the stoichiometry of the involved reaction, oxidation of 1 g lactate ion results in formation of 0.68 g acetate, indicating that the calculated yield based on the experimental data was very close to the theoretical value.

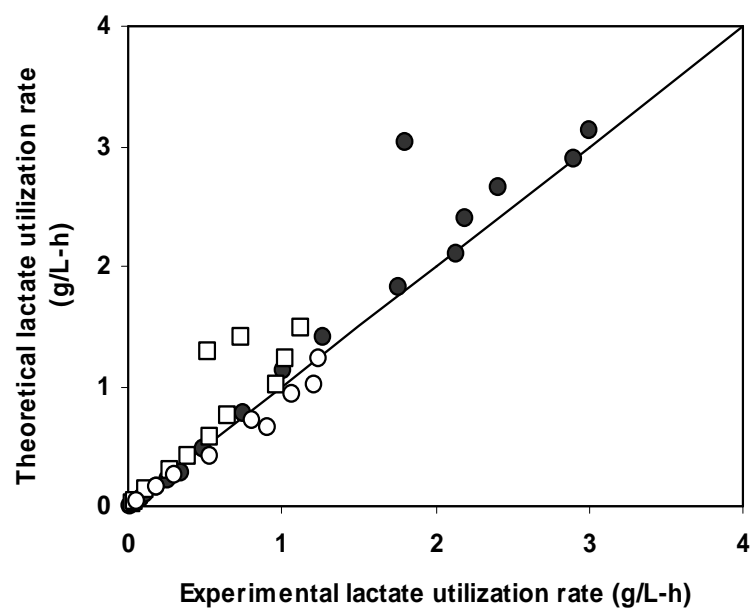


Figure 5.37 Lactate utilisation rate parity chart for the bioreactors operated with feed sulphate concentrations of 1.0, 2.5 and 5.0 g/L.
 (●)-1.0 g/L, (□)-2.5 g/L and (○)-5.0 g/L of sulphate in the feed.

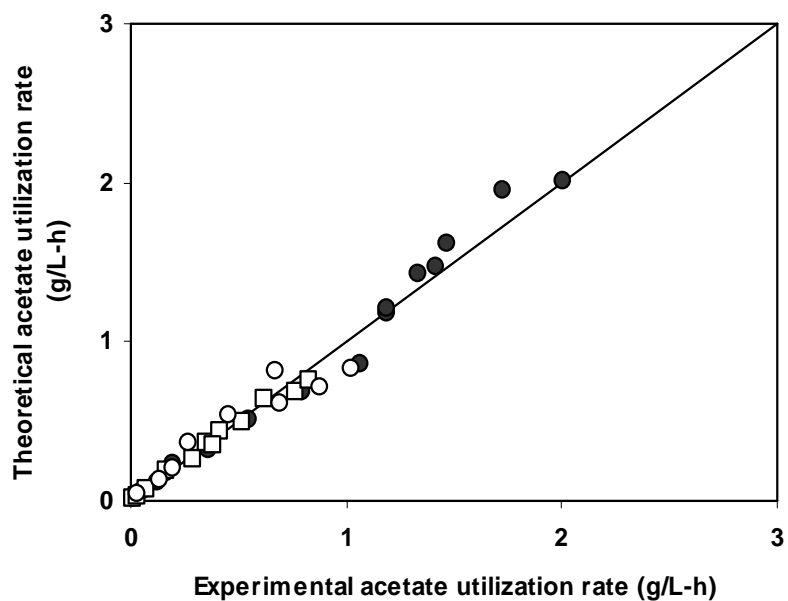


Figure 5.38 Acetate production rate parity chart for the bioreactors operated with feed sulphate concentrations of 1.0, 2.5 and 5.0 g/L.
 (●)-1.0 g/L, (□)-2.5 g/L and (○)-5.0 g/L of sulphate in the feed.

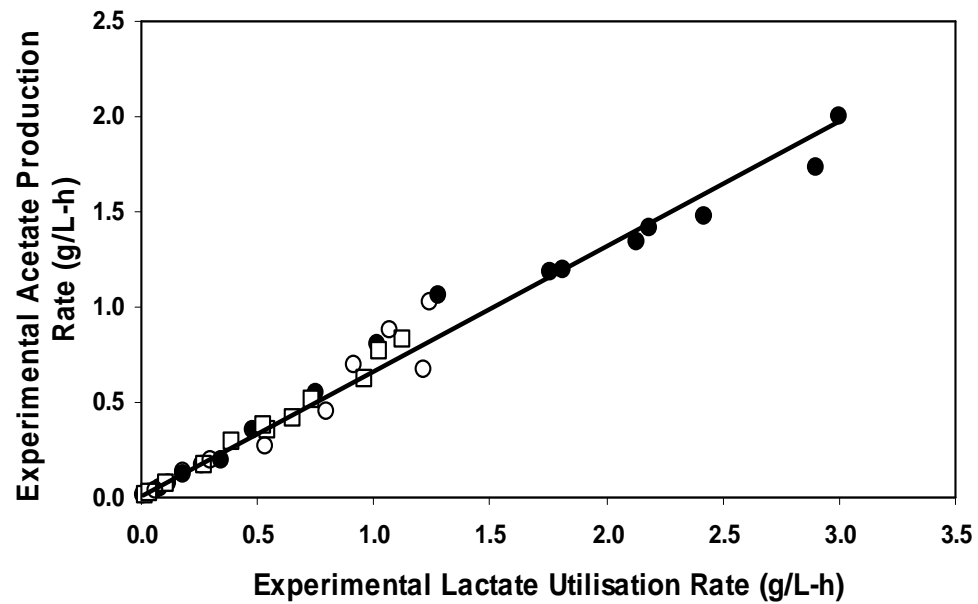


Figure 5.39 Experimental acetate production rates as a function of experimental lactate utilisation rates based on the data obtained in the bioreactors packed with sand as carrier matrix and fed with media containing 1.0, 2.5 and 5.0 g/L of sulphate. (●)-1.0 g/L, (□)-2.5 g/L and (○)-5.0 g/L of sulphate in the feed.

5.3. Comparison of Kinetic Data Reported for Freely Suspended SRB Cells and Present Results with Immobilised Cells

Using an anaerobic mixed culture, Moosa et al. (2002 and 2005) studied the kinetics of anaerobic sulphate reduction in a continuous stirred tank bioreactor. In the presence of freely suspended cells, the kinetics of reaction was shown to be dependent on the initial concentration of sulphate in the feed. An increase in initial concentration of sulphate from 1.0 to 5.0 g/L enhanced the maximum volumetric reduction rate from 0.007 to 0.075 g/L-h. Table 5.4 compares the kinetic data reported by Moosa et al. for anaerobic reduction of sulphate with freely suspended cells with those obtained in the present work for immobilized cell systems. Comparison of these data confirms the superiority of the immobilized cell system through a number of facts. First, the maximum volumetric reduction rates obtained in the present work are significantly higher than those observed in a system with freely suspended cells. Second, the corresponding residence time in the immobilized cell system is significantly shorter than in the freely suspended cell system. This indicates that the immobilized cell bioreactor has the potential for being run at higher volumetric loading rates (shorter residence times), while maintaining a high conversion and volumetric reduction rate.

The other interesting observation is the contrasting effects of feed sulphate concentration on the kinetics of reaction in the presence of immobilized and freely suspended cells. Moosa et al. (2002) reported that in the presence of the freely suspended cells, increase in feed sulphate concentration led to higher volumetric reduction rates, while in the present work lower volumetric reduction rates were achieved with immobilized cell when feed sulphate concentration was increased. One of the possible explanation for these contrasting results may lie in the fact that in the work

by Moosa et al. (2002) due to the configuration of stirred tank bioreactor, namely employment of extensive mixing and exposure of the reaction liquid to a large head space, a major portion of sulphide could have escaped from the liquid to the gas phase, thus the concentration of sulphide in the liquid phase had been below the inhibitory level. This was not the case in the present work and configuration of employed packed-bed bioreactors would not allow for the extensive transfer of sulphide to the gas phase. Unfortunately, Moosa et al. (2002 and 2005) did not report any data on concentration of sulphide either in the liquid phase or gaseous phase to confirm the validity of this postulate.

Table 5.4 Comparison of kinetic data reported for freely suspended SRB cells with those obtained with immobilized cells

Reference	State of the cells	Initial sulphate conc. (g/L)	Maximum overall reduction rate (g/L-h)	Corresponding residence time (h)	Corresponding conversion (%)
Moosa et al., 2002	Free cells	1.0	0.007	90	61.0
Moosa et al., 2002	Free cells	2.5	0.032	60	80.0
Moosa et al., 2002	Free cells	5.0	0.075	36	54.0
Present work	Immobilised cells	1.0	1.71	0.5	73.2
Present work	Immobilised cells	2.5	0.815	2.7	79.6
Present work	Immobilised cells	5.0	0.675	2.7	35.4

5.4. Comparison of the Kinetic Data Reported by Different Researchers with Those of the Present Work

Kinetic data for anaerobic reduction of sulphate as reported by different researchers are summarized in Table 5.5 and 5.6. The accurate assessment and comparison of the reported data is rather difficult. This is due to variations in microbial species and strains, experimental conditions such as sulphate concentration, pH, temperature, metal concentrations and employment of different energy sources, as well as differences in configuration of the employed bioreactors. Nonetheless, a comparison of the volumetric reduction rates was made, using the available data. Table 5.5 and Table 5.6 summarize the volumetric loading rates calculated based on void and total volumes of the bioreactors, respectively. The values indicate that the volumetric reduction rates (based on the void volume) obtained in the present work using glass beads and foam BSP are at the same level as those reported in the literature, while reduction rate obtained with the bioreactor packed with sand is higher than those reported in the literature. The volumetric reduction rates (based on the total volume) achieved in this study was very similar to those reported in the literature. The highest volumetric reduction rate (based on total volume) achieved in this study was 0.228 g/L-h for the bioreactor packed with sand and operated with a feed sulphate concentration of 1.0 g/L and which is slightly higher than the value of 0.2 g/L-h reported by Foucher et al. (2001). However, it should be pointed out that the observed reduction rate in the present work achieved at a much shorter residence time of 4.0 h, as compared to a residence time of 21.6 h employed by Foucher et al. 2001.

Table 5.5 Performance of various continuous flow packed-bed bioreactors used to treat sulphate containing waste streams reported based on their void volume

Bacterial group	Reactor type	Carrier matrix/Organic carbon source	Temp. (°C)	Feed pH	Flow rate (mL/h)	Initial sulphate concentration (g/L)	HRT (h)	Volumetric loading rate (g/L-h)	Volumetric reduction rate (g/L-h)	Reference
Mixed SRB culture	UPBR (4.78L)	Sand/lactate	25	4.5	156.6	2.5	16.2	0.155	0.02	Jong and Parry, 2003
Reactive mixtures (leaf mulch and sawdust)	UPBR (0.785L)	sand + pyrite + reactive mixture/ reactive mixture	-	6.46	-	3.66	-	-	0.005	Waybrant et al., 2002
Mixed SRB	UPBR (0.85L)	Porous ceramic carriers/methanol	-	2.9-3.2	-	2.0	12.0	0.167	0.132	Glombitza, 2000
Mixed SRB	UPBR (3.9m ³)	Crushed lava rocks/methanol	-	2.9-3.2	-	1.97	4.2	0.469	0.271	Glombitza, 2000
Spent manure	DPRB (7.85L)	Spent manure/methanol ¹	23-26	4.2	180	0.9	6.6	0.136	0.072	Tsukamoto and Miller, 1999
Mixed SRB	UPBR (2.512L)	Sand/lactate	-	4.5	36	1.484	21.8	0.068	0.031	Elliott et al., 1998
				3.25					0.026	

¹ Added methanol can reduce only 54% of sulphate; UPBR: Up-flow packed bed bioreactor; DPBR: Down-flow packed bed bioreactor; HRT: Hydraulic residence time.

Table 5.5 (Contd.) Performance of various continuous flow packed-bed bioreactors used to treat sulphate containing waste streams reported based on their void volume

Bacterial group	Reactor type	Carrier matrix/Organic carbon source	Temp. (°C)	Feed pH	Flow rate (mL/h)	Initial sulphate concentration (g/L)	HRT (h)	Volumetric loading rate (g/L-h)	Volumetric reduction rate (g/L-h)	Reference
<i>Desulfovibrio desulfuricans</i>	UPBR (1.18L)	Sand/lactate	25	7	65	0.9	6.5	0.138	0.015	Chen et al., 1993
Mixed SRB	UPBR (0.45L)	Sand/lactate	22	7	112.3	1.0	0.5	2.34	1.71	Present work
			22	7	22.5	2.5	2.7	1.02	0.815	
			22	7	22.3	5.0	2.7	1.9	0.675	
		Foam BSP/lactate	22	7	43.0	1.0	5.3	0.22	0.20	
		Glass bead/lactate	22	7	3.8	1.0	28.6	0.043	0.043	

UPBR: Up-flow packed bed bioreactor; DPBR: Down-flow packed bed bioreactor; HRT: Hydraulic residence time.

Table 5.6 Performance of various continuous flow packed-bed bioreactors used to treat sulphate containing waste streams reported based on the total volume of the bioreactor.

Bacterial group	Reactor type	Carrier matrix/Organic carbon source	Temp. (°C)	Feed pH	Flow rate (mL/h)	Initial sulphate concentration (g/L)	HRT (h)	Volumetric loading rate (g/L-h)	Volumetric reduction rate (g/L-h)	Reference
Mixed SRB	UPBR (21.58L) ¹	Special packing / H ₂ andCO ₂ + sodium acetate	30	2.55	900	5.8	21.6	0.269	0.20	Foucher et al., 2001
Digested sludge	DPBR (42.65L) ¹	Plastic ballast rings/ acetate	35	7	708.3	0.9	60	0.015	0.013	Lin and Lee, 2001
Mixed acidophilic or neutrophilic SRB	UPBR (2L)	Porous glass beads/ethanol+ lactic acid+ glycerol	-	4	40	1.64	49.3	0.03	0.022	Kolmert and Johnson, 2001
Anaerobic digester fluid	UPBR (0.20L)	SWP/SWP	25	6.8	0.4	2.58	480	0.005	0.005	Chang et al., 2000
Mixed SRB	UPBR (0.45L)	Sand/lactate	22	7	112.3	1	4.01	0.312	0.228	Present work
			22	7	22.5	2.5	20	0.136	0.108	
			22	7	22.3	5	20.18	0.25	0.09	
		Foam BSP/lactate	22	7	43.0	1	10.46	0.115	0.101	
		Glass bead/lactate	22	7	3.8	1	117.18	0.01	0.01	

¹Effluent recycle; UPBR: Up-flow packed bed bioreactor; DPBR: Down-flow packed bed bioreactor; SWP: sludge from wastepaper recycling plant; HRT: Hydraulic residence time.

5.5. Reproducibility of the Kinetic Data

The reproducibility of the kinetic data was investigated by repeating the experimental run with sand as a carrier matrix and a medium containing 1.0 g/L sulphate. Steady state volumetric reduction rate as a function of sulphate volumetric loading rate for these two experimental runs are compared in Figure 5.40. Unfortunately and due to a technical problem (appearance of a crack in the bioreactor glass column and leakage of the liquid), running of the second bioreactor for the entire range of volumetric loading rate was not possible and the second experimental run was performed up to a volumetric loading rate of 0.56 g/L-h (residence time: 1.8 h). However, comparison of the collected data confirmed that the kinetic data could be reproduced with high accuracy.

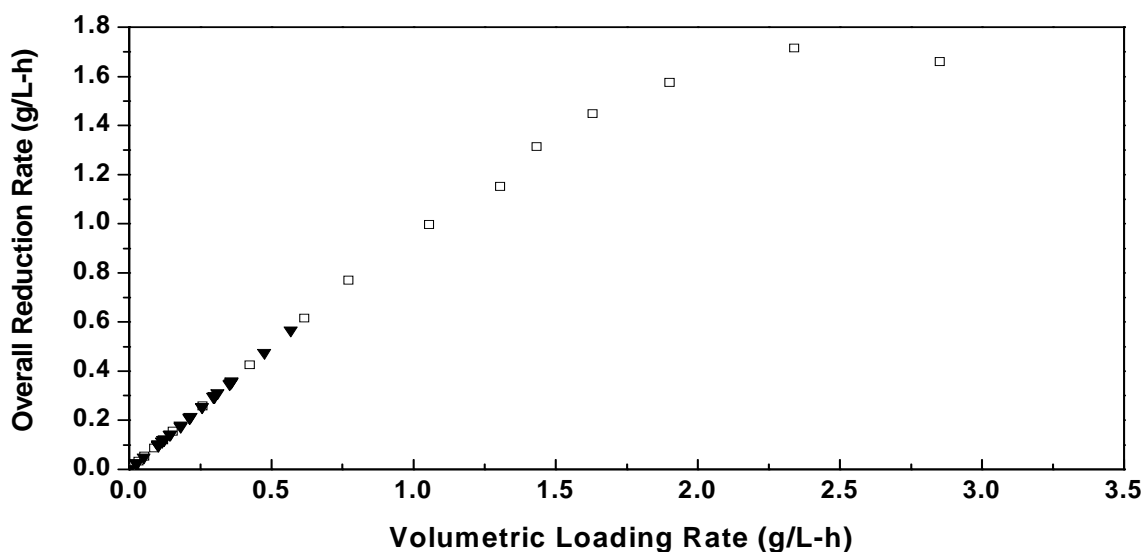


Figure 5.40 Volumetric reduction rate profiles for two independent experimental runs in the bioreactor fed with 1.0 g/L of sulphate and packed with sand
(□)-Initial run and (▼)-Repeat run.

6. CONCLUSIONS AND RECOMMENDATIONS

The effects of carrier matrix, sulphate initial concentration and volumetric loading rate on the kinetics of sulphate reduction were investigated in immobilized cell bioreactors. The major findings of this work are summarised in this chapter.

6.1. Conclusions

- In the presence of immobilized cells, volumetric reduction rate passed through a maximum as volumetric loading rate was increased.
- The extent of maximum volumetric reduction rate and the corresponding residence time were dependent on the employed carrier matrix, specifically the total surface area which the carrier matrix provided for the establishment of biofilm. Among the three tested carrier matrices, sand displayed a superior performance and a maximum volumetric reduction rate of 1.7 g/L-h was achieved at the shortest residence time of 0.5 h. This volumetric reduction rate was 40 and 8 folds faster than the volumetric reduction rates obtained with glass beads (0.04 g/L-h; residence time: 28.6 h) and foam BSP (0.2 g/L-h; residence time: 5.3 h), respectively.

- The reduction of sulphate was coupled to incomplete oxidation of lactate. The experimentally determined values of lactate utilization and acetate production rates were in close agreement with those predicted based on the stoichiometry of the involved reactions.
- Concentration of sulphate in the feed influenced the kinetics of sulphate reduction and higher volumetric reduction rates were observed with the lowest concentration of sulphate in the feed (1.0 g/L). When feed sulphate concentration was increased from 1.0 to 5.0 g/L, the maximum volumetric reduction rate decreased from 1.71 to 0.675 g/L-h.
- Bioreactors packed with sand as a carrier matrix showed a robust performance over the wide range of applied volumetric loading rate and for the entire period of experimental runs (119 to 224 days).
- Kinetics of anaerobic sulphate reduction in immobilized cell bioreactors were significantly faster than those reported for stirred tank bioreactors with freely suspended cells.
- Based on a repeated experimental run, the kinetic data obtained in this work were reproducible.

6.2. Recommendations for Future Work

The following section presents the recommendations for future work.

- Kinetics of anaerobic sulphate reduction should be studied in an immobilized cell bioreactor, using other carbon and energy sources such as acetate, propionate, butyrate, pyruvate, and specifically a combination of hydrogen and CO₂ and waste materials to find out the most economical and environmental friendly carbon and energy source.
- Effects of temperature and pH on the kinetics of sulphate reduction should be studied. The possibility of using acid tolerant and psychrophilic strains of SRB should be explored.
- Inhibitory effects of metals which are usually present in the AMD and also sulphide on the kinetics of sulphate reduction should be studied in an immobilised cell bioreactor.
- Performance of the immobilized cell bioreactor utilized for anaerobic sulphate reduction should be simulated mathematically, using the available rate expression for sulphate reduction and the kinetic data obtained from this study.

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APPENDICES

A: Calibration Curves for Sulphide, Sulphate and Protein Measurements

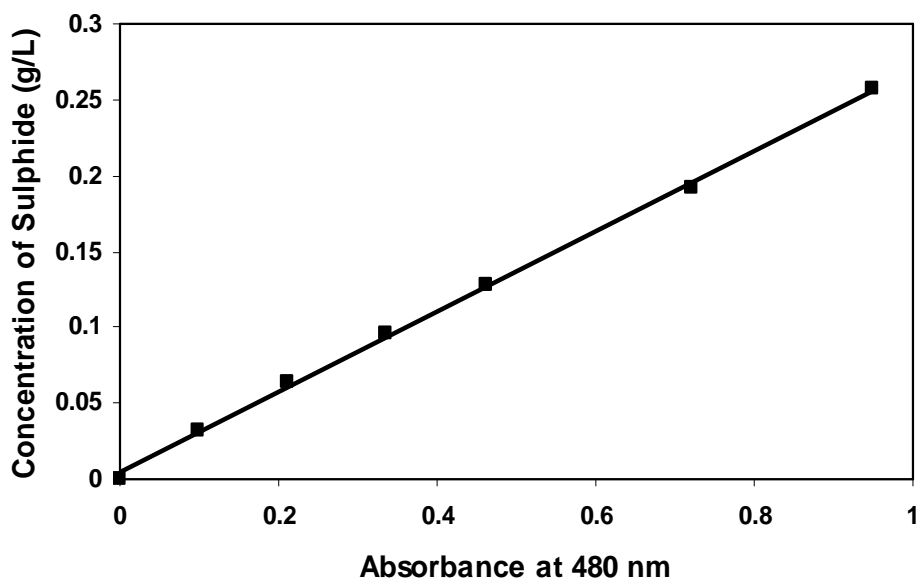


Figure A.1 Calibration curve for sulphide determination

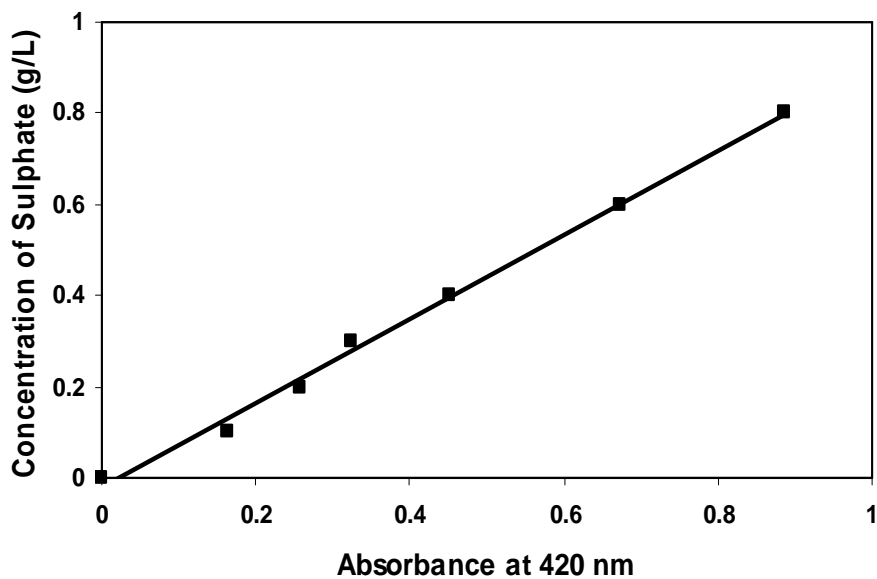


Figure A.2 Calibration curve for sulphate determination

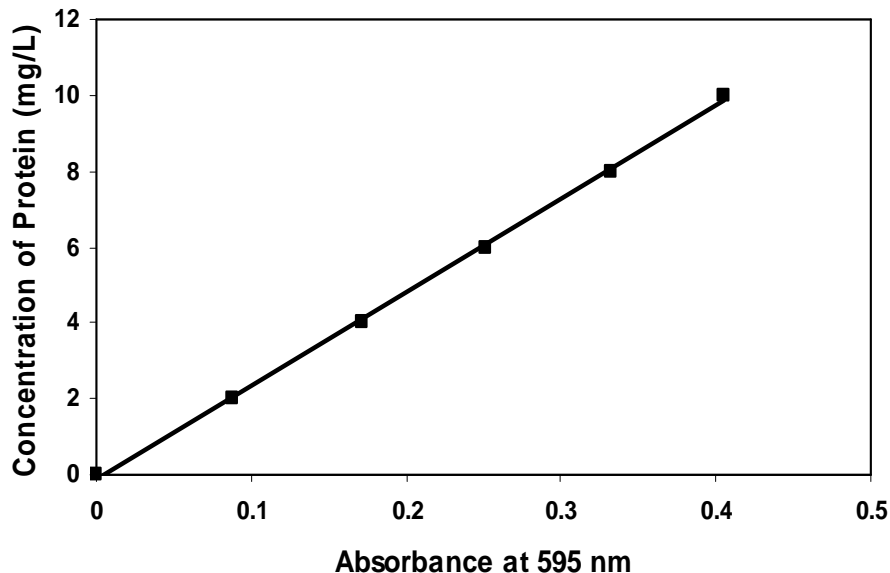


Figure A.3 Calibration curve for protein measurement

B: Research Contributions

- **Vikrama K.**, Handojo M. and Nemati M. (2004). Biotreatment of a Sulfate-Containing Waste Stream in an Anaerobic Up-Flow Packed-Bed Bioreactor. *1st Water and Environment Specialty Conference of the Canadian Society for Civil Engineering*, Saskatoon, Canada.
- Poster presentation at 2004 Canadian Society for Civil Engineering conference (CSCE), Saskatoon, SK, Canada.
- Oral presentation at 55th Canadian Society for Chemical Engineering conference (CSCHE, 2004), Calgary, AB, Canada.
- **Vikrama B.** and Nemati M. (2005). Kinetics of Anaerobic Sulphate Reduction in immobilised cell bioreactors: Effects of concentration and volumetric loading rate of sulphate. *Biochemical Engineering Journal*. Submitted.